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**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
CENTER FOR FOOD SCIENCE AND NUTRITION**

**Effect of Germination and Fermentation on Microflora, Sensory
Characteristics and Energy Density of Fermented Low Tannin
Sorghum/Chickpea Blended Porridge as Complementary Food**

By

Tsion Markos

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

**Advisors: Mr. Kelbessa Urga
Mr. Tilahun Bekele**

**June, 2013
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Approved by Examining Board:

Name	Signature	Date
Mr. Kelbessa Urga (Advisor)		109/07/2013/
Mr. Tilahun Bekele (Advisor)		103/07/2013/
Dr. Gulelat Dessie (Examiner)		103/07/2013/
Dr. Melaku Umeta (Examiner)		/ / / /
Mr. Ashagrie Zewdu (Chairman)		103/07/2013/

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Acronyms

AAU	Addis Ababa University
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
DZARC	Debre Zeit Agricultural Research Centre
EHNRI	Ethiopian Health and Nutrition Research Institute
EIAR	Ethiopian Institute of Agricultural Research
ESE	Ethiopian Seed Enterprise
FAO	Food and Agricultural Organization
HU	Hawassa University
LAB	Lactic Acid Bacteria
MRS	Man, Rogosa and Sharp
PAHA	Pan American Health Organization
PCA	Plate Count Agar
SPSS	Statistical Package for Social Science
TA	Titrateable Acidity
USA	United States of America
USDA	United Nations Department of Agriculture
NUICEF	United Nations International Children's Fund
WFP	World Food Program
WHO	World Health Organization
RNI	Recommended Nutritional Intakes

Abstract

*Nutritional composition, microbiology and sensory characteristics of germinated and fermented low tannin sorghum (*Sorghum bicolor* L. Moench) variety ("teshale") and kabuli chickpea (*Cicer arietinum* L.) variety ("harbu") blend were investigated. Traditional processing (germination and fermentation) methods were observed to be effective in reducing antinutritional factors. Standard Methods of AOAC were used for analysis of nutritional compositions. Porridges were evaluated for their acceptability test using a nine point hedonic scale. Total plate count and lactic acid bacterial count were enumerated using Plate Count Agar and MRS agar, respectively. Fermentation for 48 hours reduced the total bacterial count from 9.57 to 3.16logcfu/m by reducing the pH to the level of <4.5 which inhibited the growth of total bacterial count to the level <4logcfu/ml. Combined effect of germination and fermentation increased crude protein from 13.55 to 15.78, total energy from 410.32 to 415.11, calcium from 51.49 to 57.76, zinc from 2.676 to 3.17 and phosphorus from 26.11 to 38.87 mg/100g, but decreased crude fiber from 3.05 to 2.47, carbohydrate from 78.90 to 76.74, iron from 5.29 to 4.28, phytate from 52.02 to 20.10 and tannin from 38.35 to 34.19 mg/100g of sorghum/chickpea blend. Over all sensory acceptability of fermented/germinated sorghum/chickpea blended porridge was more liked by the panelists. Combined effect of germination/fermentation increased the energy density of sorghum/chickpea blend by reducing viscosity from 5122cP (control) to 1986cP (acceptable value of viscosity for young children). It can be concluded that, germination and fermentation were observed to decrease the total bacterial count, increase energy density by decreasing the viscosity of porridge and increase the sensory acceptability by improving the organoleptic property of porridge prepared from low tannin sorghum/chickpea blend.*

Key words: Germination, Fermentation, Complementary food, Antinutritional factors, Low tannin sorghum, Chickpea.

Chapter One

1. Introduction

1.1. Background

In many developing countries, porridges used as complementary foods for young children are generally prepared from cereals or are mixed with legumes in water. The dry matter concentration of traditionally prepared complementary foods by mothers or at small-scale in traditional production units is not generally sufficient to provide the energy and the nutrients necessary to meet the nutritional requirements of young children (Sanni, Onilude, and Ibidapo, 1999; WHO/NUT, 1998) which is below the values recommended by Dewey and Brown (2003) for complementary foods. Consequently, these result in under-nutrition of the infant and young children.

Sorghum is an important staple food, particularly in semi-arid tropical regions of Africa and Asia (Balole, 2005). It is commonly known that the main nutritional drawback sorghum, is their low protein content and the limited biological quality of their protein (highly deficient in lysine and tryptophan) compared to animal protein. Nevertheless, the protein quality can be improved by combining it with other rich sources of protein (Joseph, 2009). The tannin content of sorghum also affects its nutritional value. High- and low-tannin sorghum types are distinguished. High-tannin sorghum types (sometimes called 'brown sorghums', although the grain may also be white, yellow or red) have less nutritional value but have agronomic advantages, including resistance to birds, insects, fungi and decreased sprouting in the panicle. Sorghum types without a pigmented grain wall ('white sorghums') do not contain condensed tannins and have a nutritional value similar to that of maize (USDA, 2004).

Chickpeas are one of the oldest and most widely consumed legumes in the world, particularly in tropical and subtropical areas. Chickpea has significant amounts of all the essential amino acids except sulfur containing types, which can be complemented by

adding cereals to daily diet. Lipids are present in low amounts but chickpea is rich in nutritionally important unsaturated fatty acids like linoleic and oleic acid.

Germination and fermentation treatments have been employed by various communities to enhance organoleptic properties of foods. Germination increases the endogenous phytase activity in cereals, legumes, and oil seeds through de Novo synthesis, activation of intrinsic phytase or both leading to reduction of total phytates in the cereals thus enhancing their nutritional value. Another beneficial effect of germination is the increase in the activity of α -amylase enzyme of cereals, especially in sorghum and millet. The complex carbohydrates are hydrolyzed to dextrans and maltose, reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Gibson et al., 1998).

Fermented food has many beneficial product effects on food. These include biomass proteins and amino acids, vitamins, minerals, carbohydrates, flavor and aroma compounds. These alter the pH of foods to levels that reduce the growth of pathogenic microorganisms. Fermentation therefore, not only enhances food safety and shelf life, naturally preserving the food, but also enhances nutritional value (Gibson et al., 1998).

Adequate nutrition is essential for children's health and development. But, inadequate intake of protein in developing countries has led to various forms of malnutrition in children. It has been reported that in developing countries; protein malnutrition persists as a principal health problem among children below the age of five. Under nutrition is also a major cause of disability preventing children who survive from reaching their full development potential. Child undernutrition is associated with shorter adult height, lower level of academic achievement, reduced adult income, and low birth of their pregnancy (EHNRI, 2010). The incidence of malnutrition rises sharply during the period from 6 to 18 months of age in most countries, and the deficits acquired at this age are difficult to compensate for later in childhood (WHO, 2002).

Supplementation of cereals with locally available legumes rich in protein increases the protein content of cereal-legume blends and their protein quality through mutual complementation of their individual amino acids (Joseph, 2009). For example, Joseph (2009) demonstrated that mixing sorghum with cowpea at ratio of 70:30 (w/w) improved nutritional quality of fermented gruel (Ogi) substantially. But, there is information gap on effect of germination and fermentation specifically on sorghum/chickpea blend as to enhance nutritional composition, hygienic condition and organoleptic characteristics as complementary childhood food preparation. The potential of blending sorghum with chickpea in Ethiopian child nutrition has generally been underestimated and there are also knowledge gap to appropriate blending and processing these grains to the wide use in the country. So that the current study focus on the effect of germination and fermentation on energy density, microflora and sensory characteristics of low tannin sorghum variety blending with chickpea to prepare complementary children porridge.

1.2. Statement of the problem

Poor complementary feeding practices contribute to malnutrition. The Ethiopian DHS 2005 found that in Ethiopia, one-fourth of children between 9–23 months of age were not receiving complementary foods. Those who were fed complementary foods received too few meals, and these foods lacked critical micronutrients. Ethiopian Demographic and Health Survey (2005) show that Forty-seven percent of children under five are stunted and 24 percent are severely stunted. Eleven percent of children under five are wasted and 2 percent are severely wasted. The weight for age indicator shows that 38 percent of children under five are underweight and 11 percent are severely underweight (EDHS, 2005). Because of this, in Ethiopia it is estimated that under-nutrition is responsible, directly or indirectly for 53% of all under 5 mortality (WHO, 2002).

The main dietary sources of iron and zinc in Ethiopia are cereals and legumes which are of low energy density and contain antinutritional factors that reduce the bioavailability of nutrients (Melaku, 2005). Sorghum have low protein content and limited biological quality of their protein (highly deficient in lysine and tryptophan) but rich in methionine and threonine, and chickpea is deficient in sulfur containing amino acid (methionine and

cysteine) but rich in lysine and tryptophan. When blended, they complement the essential amino acids which are limited to one other. There is also high content of antinutritional factors which limits the use these foods as complementary food.

Different technological methods have been tested to increase the energy density and nutritional composition of cereal/legume based complementary foods for young children. Drum drying and extrusion cooking, are either too complicated or too expensive for low-income families in Ethiopia. Thus, practical, low-technology processes for production of complementary foods with adequate paste viscosity and nutrient density are needed. The present work aimed to increase the energy density of sorghum based complementary food by blending with chickpea and processing using traditional processing methods (germination and fermentation) to minimize the anti-nutritional factors and improve the nutritional bioavailability. These processes are chosen according to their potential to favor partial starch hydrolysis (germinated cereals and fermentation) and also to improve hygienic conditions (fermentation).

1.3. Significance of the study

The results of this study will have the following significances:

- ❖ It provides necessary information about blending, germination and fermentation of low tannin sorghum/chickpea blend, resulting in improved high energy density complementary food.
- ❖ It will contribute in overcoming prevalence of malnutrition with providing nutritionally rich and anti-nutritionally low white sorghum/chickpea blended complementary food.
- ❖ It can be used as a base line data for further research in the same area to compare nutritional status of white sorghum/chickpea blended flour with other varieties of sorghum/chickpea and also new product development by using sorghum/chickpea flour.
- ❖ It can be used as reference material for students, teachers and other researchers in related areas.

1.4. Hypothesis of the study

H₀: - Germination and fermentation have no effect on the sensory characteristics, microflora and energy density of germinated and fermented porridges from low tannin sorghum/chickpea blend as complementary food.

H₁:- Germination and fermentation have significant effect on the sensory characteristics, microflora and energy density of germinated and fermented porridges from low tannin sorghum/chickpea blend as complementary food.

1.5. Objectives of the study

1.5.1. General objectives

The general objective of this study was to investigate the effects of germination and fermentation on the sensory characteristics, microflora and energy density of fermented porridge from low tannin sorghum/chickpea blend as complementary food.

1.5.2. Specific objectives

The specific objectives of this study are:

1. To evaluate the microbiological characteristics during sorghum/chickpea fermentation.
2. To assess the effect of germination and fermentation on nutritional composition of the sorghum/chickpea blend.
3. To investigate the effect of germination and fermentation on the energy density of sorghum/chickpea blend.
4. To analyze the sensory characteristics of the germinated and fermented sorghum/chickpea porridges.

1.6. Limitation of the study

The conclusion made in this study was only on low tannin sorghum cultivar (“Teshale”) and Kabuli chickpea cultivar (“Harbu”), because of availability of only these samples in the research center during sample collection. So, this study needed further research to

make conclusions including other low tannin sorghum and chickpea variety. Furthermore, it might be better if other important parameters including amino acid profiles, vitamins complex and potential to fortify mineral were involved in this study, but shortage of time and money did not pave the way to me to conduct additional experimentation beyond the selected activities. During laboratory attachment of the study, absence of some laboratory equipment at the Center for Food Science and Nutrition was another challenge.

Chapter Two

2. Literature Review

2.1. Nutrition and child health

Adequate nutrition during infancy and early childhood is fundamental to the development of each child's full human potential. It is well recognized that the period from birth to two years of age is a "critical window" for the promotion of optimal growth, health and behavioral development. After a child reaches two years of age, it is very difficult to reverse stunting that has occurred earlier (WHO, 2001). The immediate consequences of poor nutrition during these formative years include significant morbidity and mortality and delayed mental and motor development. In the long term, early nutritional deficits are linked to impairments in intellectual performance, work capacity, reproductive outcomes and overall health during adolescence and adulthood. Thus, the cycle of malnutrition continues, as the malnourished girl child faces greater odds of giving birth to a malnourished, low-birth-weight infant when he/she grows up.

Poor infant feeding practices, coupled with high rates of infectious diseases are the principal proximate causes of malnutrition during the first two years of life. For this reason, it is essential to ensure that caregivers are provided with appropriate guidance regarding optimal feeding of infants and young children. However, there are a number of infants who will not be able to enjoy the benefits of breastfeeding in the early months of life or for whom breastfeeding will stop before the recommended duration of two years or beyond. A group that calls for particular attention is the infants of mothers who are known to be HIV-positive. To reduce the risk of transmission, it is recommended that when replacement feeding is acceptable, feasible, affordable, sustainable and safe, mothers should avoid breastfeeding from birth. Otherwise, they should breastfeed exclusively and make the transition to exclusive replacement feeding as soon as alternative feeding options become feasible. Other circumstances that may prevent a child from being breastfed include death or severe illness in the mother, or inability or lack of desire by the mother to breastfeed (WHO, 2010).

2.2. Nutritional status of children in Ethiopia

The 2005 EDHS included information on the nutritional status of children under five years of age for three indices, namely, weight-for-age, height-for-age and weight-for-height, taking age and sex into consideration. According to the data, forty-seven percent of children under five are stunted and 24 percent are severely stunted. Eleven percent of children under five are wasted and 2 percent are severely wasted. The weight for age indicator shows that 38 percent of children under five are underweight and 11 percent are severely underweight. Rural children are more stunted (48 percent) than urban children (30 percent). Regional variation in nutritional status of children is substantial. Stunting levels are above the national average in the Amhara (57 percent) and SNNP (52 percent) regions. The proportion of children wasted is higher in rural areas (11 percent) than in urban areas (6 percent). The percentage of children underweight increases sharply from 4 percent among children under age 6 months to 19 percent among children age 6-8 months, doubles among children age 9-11 months, and peaks at 48 percent among children age 12-23 months with very small decreases thereafter. This may be due to inappropriate and/or inadequate feeding practices because increasing levels of children underweight by age coincides with the age at which normal complementary feeding starts (EDHS, 2005).

2.3. Germination

Germination is a natural process occurred during growth period of seeds in which they meet the minimum condition for growth and development. During this period, reserve materials are degraded, commonly used for respiration and synthesis of new cells prior to developing embryo. The process starts with the uptake of water by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radical (Megat Rusydi, et al., 2011). Steps in seed germination are: - (i). The seed absorbs water and seed coat bursts. It is the first sign of germination. There is an activation of enzymes, increase in respiration, and plant cells get duplicated. A chain of chemical changes starts which leads to the development of the plant embryo. (ii). Chemical energy stored in the form of starch is converted to sugar, which serves as food for the embryo during the

germination process. Soon, the embryo gets nourished and enlarged, and the seed coat bursts open. (iii). The growing plant emerges out of the seed. Tip of the root first emerges, growing downwards, and helps to anchor the seed in place. It also allows the embryo to absorb minerals and water from soil. (iv). Some seeds require special treatment of temperature, light or moisture to start germination (Buzzle, 2013).

Processing techniques such as germination and fermentation have been found to improve the quality of cereals due to chemical changes that enhance organoleptic response (Nou, 1992), contents of free sugars, protein and vitamins, as well as bioavailability of minerals (Zamora and Fields, 1979) and results in the breakdown of some of the anti-nutritional endogenous compounds (Ahmed et al., 2006). In many instances, usage of only one method may not impart the desired removal of anti-nutritional compounds and a combination of two or more methods is required (Hassan et al., 2007).

2.3.1. Nutritional value of germinated foods

Germination has profound effect on nutritional quality of the cereal (Chavan and Kadam, 1989b). During germination, certain changes occur in terms of quantity and type of nutrients within the seed. These changes can vary depending on the type of vegetable, the variety of seed and the condition of germination (Bau et al., 1997). Several studies have shown that germination improves the nutritive value of cereals and legumes. Germination has been found to decrease the levels of antinutrients present in cereals and maximizes the levels of some of the utilizable nutrients (Inyang and Zakari, 2008). According to Sangronis and Machado (2007) in (Megat Rusydi, and Azrina, 2012) germinated seeds are good source of ascorbic acid, riboflavin, choline, thiamine, tocopheroles and pantothenic acid.

2.4. Fermentation

2.4.1. Historical perspectives of fermentation

The process of fermentation has been practiced for millennia resulting in tremendous variety of fermented foods ranging from those derived from meat and plants to those derived from milk and dairy products. Traditionally, fermented foods have contributed

greatly to both variety and safety of the human diet. Fermentation can be traced back thousands of years and has been used as a means of improving the keeping quality of food for more than 6000 years. The essential role of microorganisms in the fermentation process was realized in 1861 A.D during development of pasteurization (Klaenhammer and Fitzgerald, 1997). Together with drying and salting, fermentation is one of the oldest methods of food preservation. Its importance in modern day life is underlined by the wide spectrum of fermented foods marketed both in developing and industrialized countries; not only for the benefits of preservation and safety, but also for their highly appreciated sensory attributes (Ramaite, 2004).

Fermentation has enabled our ancestors in temperate and cooler regions to survive winter season and those in the tropics to survive drought periods, by improving the shelf life and safety of foods (Campbell-Platt 1994). Through the age, fermentation has had a major impact on the nutritional habits and tradition, on culture and also on the commercial distribution and storage of food. Traditional fermentation still serves as a substitute where refrigeration or other means are not available for the safe keeping of food. However, food technologists are convinced that they are able to improve traditional fermentation processes. This is based on their scientific understanding of the fermentation processes and their expertise in breeding and in microorganisms for strain improvement and the use of pure starter culture (Ramaite, 2004)..

They have, however, proven the ability to influence the duration of fermentation processes and the taste and value of the end products. Processing usually involves either soaking of the raw materials, submerged in water contained a fermenting vat (usually clay pots) for a length of time or an initial size reduction of the raw material by grating or milling in the wet form before being allowed to ferment without being soaked in water (Ramaite, 2004).

2.4.2. Benefits of fermentation

Fermented foods may be generally described as palatable and wholesome foods, prepared from raw or heated materials by microbial fermentation. Microorganisms, by virtue of

their metabolic activities, contribute to the development of characteristic properties such as taste, aroma, visual appearance, texture, shelf life and safety. Fermentation is also one of the processes that decreases the level of antinutrients in food grains and increases the starch digestibility, protein digestibility and nutritive value. The nutritional evaluation of fermented grains has been examined by many workers. Fermentation also leads to an increase in protein content, enhancement of carbohydrate accessibility, improvement in amino acid balance, decrease in antinutritional factors like tannin and phytic acid (Alka et al., 2012). It is known that pathogenic microorganisms normally found in food will not be able to grow in an acid environment that is at pH below four. This acidity is normally found in lactic acid fermented food (Sahlin, 1999).

According to Nout (1994) explained in (Ramaite, 2004) fermentation plays at least five roles in food processing:

- ✓ Enrichment of the human dietary through development of a wide diversity of flavors, aromas and textures in food:
- ✓ Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid, alkaline fermentations and high salt fermentations;
- ✓ Enrichment of food substrates biologically with vitamins, protein, essential amino acids and essential fatty acids:
- ✓ Detoxification during food fermentation processing and
- ✓ A decrease in cooking times and fuel requirements.

2.4.3. Nutritional value of fermented foods

Generally, a significant increase in the soluble fraction of a food is observed during fermentation. The quantity as well as quality of the food proteins as expressed by biological value, and often the content of water soluble vitamins is generally increased, while the antinutritional factors show a decline during fermentation. Fermentation results in a lower proportion of dry matter in the food and the concentrations of vitamins, minerals and protein appear to increase when measured on a dry weight basis (Adams 1990). According to the Wakil and Kazeem (2012) the analysis of the proximate composition of all the starter fermented formulated sorghum-cowpea blends indicates that

the protein content rose with increase in fermentation time while the moisture content, ether extract, ash content, crude fiber and total carbohydrate content decreased with fermentation time. Single as well as mixed culture fermentation of pearl millet flour with yeast and lactobacilli significantly increased the total amount of soluble sugars, reducing and non-reducing sugar content, with a simultaneous decrease in its starch content.

Combination of cooking and fermentation improved the nutrient quality of all tested sorghum seeds and reduced the content of antinutritional factors to a safe level in comparison with other methods of processing. The levels of vitamin B12, riboflavin and folacin were increased by lactic acid fermentation of maize flour, while the level of pyridoxine was decreased. A study on the effect of fermentation of cowpea on the nutritional quality of the cowpea meal showed that 72h fermentation increased the content of protein, ash and lipid levels while decreasing the levels of tannin and phytate (Sahline, 1999).

2.5. Complementary Foods

2.5.1. Concepts and definitions of complementary foods

According to current UN recommendations, infants should be exclusively breastfed for the first six months of life, and thereafter should receive appropriate complementary feeding with continued breastfeeding up to two years or beyond. Complementary food is any food other than breastmilk given in the complementary feeding period (6-24 months). Foods, given in addition to breast milk, are called complementary foods. The process of feeding them is called complementary feeding.

Complementary foods can be especially prepared for the infant or can be the same foods available for family members, modified in order to meet the eating skills and needs of the infant. In the first case, they are called transitional foods, and in the second case, there is no specific nomenclature. The terms weaning foods and supplementary feeding, widely used for a long time, are not recommended as synonyms for complementary feeding, since their use is incorrect, giving the impression that foods are introduced to replace

breastmilk, instead of complementing it. The use of the term weaning is not advisable, since in many countries, it may be understood as total cessation of breastfeeding and cause problems in breastfeeding promotion. The term weaning was used to indicate the transition between exclusive breastfeeding and the cessation of breastfeeding. Nowadays, the term full weaning is used to indicate the total cessation of breastfeeding (Cristina et al., 2004).

2.5.2. Nutritional quality of complementary food

The latest estimated energy requirements from complementary foods, assuming an average breast-milk intake, are 200 kcal /day for infants aged 6–8 months, 300 kcal/ day for infants' aged 9–11 months, and 550 kcal/day for children aged 12–23 months (WHO/UNICEF, 1998). Table 2.1 shows the WHO-recommended number of meals or snacks that children should receive daily and the associated amount of energy needed from complementary foods, depending on whether the infant is breastfed or not. Achieving these energy intakes requires that both feeding frequency and energy density of complementary foods be adequate. An energy density of <0.6 kcal g⁻¹ is generally considered low.

Table 2.1 Recommendations for feeding frequency (meals or snacks) of complementary foods according to child's age and breastfeeding status and energy needed from complementary foods for breastfed and non-breastfed infants or young children in developing countries (WHO and PAHO, 2009) , and estimated gastric capacity

Age of child (months)	Recommended daily feeding frequency		Energy needs from complementary foods		Gastric capacity (mL/meal) α	
	Breastfed	Not breastfed	Breastfed β (kcal/day)	Not breastfed (kcal/day)	Average child	Growth retarded Child
6-8	2-3	4-5	200	600	249	192
9-11	3-4	4-5	300	700	285	228
12-23	3-4	4-5	550	900	345	273

α . Assuming body weights of 8.3, 9.5, and 11.5 kg for well-nourished children and 6.4, 7.6, and 9.1 kg for growth retarded children among children 6 to 8, 9 to 11, and 12 to 23 months of age, respectively, and gastric capacity of 30 g/kg body weight.

β . Assuming average breastmilk intake

However, a comparison of average nutrient intakes of children aged 6-24 months and new dietary reference intakes published by the Institute of Medicine, USA shows that the diets of infants and young children in most populations in low-income countries are consistently deficient in some nutrients, including iron, zinc and vitamin B6. Adequate energy and nutrient intakes for this age-group are the result of a balance between appropriate breastfeeding and complementary feeding. Support for sustained breastfeeding as part of efforts to improve complementary feeding is critical. Increasing complementary feeding frequency, for example, may impair breast milk intake with the potential risk of reducing total energy and nutrient intake if not enough attention is paid to sustaining breastfeeding.

The amount of protein needed from complementary foods increases from about 2 g/ day at 6–8 months to 5–6 g day⁻¹ at 12–23 months, with the percentage from complementary foods increasing from 21% to about 50% (Dewey 2008). There is uncertainty about the optimal intake of fat during the first 2 years of life. Breastmilk is usually rich in fat (approximately 30–50% of energy), so little additional fat from complementary foods is needed while breastmilk intake is still high. However, the fat content of complementary foods becomes more important as breastmilk intake declines with age. To achieve at least 30% of energy from fat in the total diet, the amount of fat needed from complementary foods (assuming average breastmilk intake) is zero at 6–8 months, approximately 3 g/ day at 9–11 months and 9–13 g/ day at 12–23 months, or 0%, 5–8% and 15–20% of the energy from complementary foods respectively (Dewey, 2005) (a range is given because of variability in breastmilk fat concentration) (Dewey and Adu-Afarwuah, 2008).

Complementary foods should be varied and include adequate quantities of meat, poultry, fish or eggs, as well as vitamin A-rich fruits and vegetables every day. Where this is not possible, the use of fortified complementary foods and vitamin mineral supplements may be necessary to ensure adequacy of particular nutrient intakes. As infants grow, the consistency of complementary foods should change from semisolid to solid foods and the variety of foods offered should increase. By eight months, infants can eat ‘finger foods’

and by 12 months, most children can eat the same types of food as the rest of the family (WHO, 2002).

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

2.6.1. Distribution of sorghum in the world

Sorghum grain is the fifth most important cereal in the world after wheat, rice, maize and barley. In Africa it comes second after maize in terms of production. According to FAO estimates, the average world production of sorghum grain in 1999–2003 amounted to 57.7 million tons/year from 42.6 million hectare. The production in sub-Saharan Africa was 19.0 million tons/year from 22.8 million hectares. The main producing countries are the United States, India, Nigeria, Mexico, Sudan, Australia, Ethiopia and Burkina Faso. In sub-Saharan Africa annual production increased from around 10 million tons from 13 million hectares in the early 1960s to about 20 million tons from 25 million hectares in the early 2000s. In tropical Africa most sorghum is grown for home consumption, except for beer production (USDA, 2004).

In terms of tonnage, sorghum is Africa's second most important cereal. The continent produces about 20 million tonnes of sorghum per annum, about one-third of the world crop. Sorghum originated in Africa. It is uniquely adapted to Africa's climate, being both drought resistant and able to withstand periods of water-logging. Sorghum in Africa is processed into a very wide variety of attractive and nutritious traditional foods, such as semi-leavened bread, couscous, dumplings and fermented and non-fermented porridges. It is generally, although not universally considered to have first been domesticated in North Africa, possibly in the Nile or Ethiopian regions as recently as 1000 BC. World annual sorghum production is over 60 million tonnes, of which Africa produces about 20 million tonnes. This makes sorghum, quantitatively the second most important cereal grain in Africa after maize. Sorghum production takes places across the continent, with the northern African countries of Nigeria, Sudan, Ethiopia and Burkina Faso accounting for nearly 70% of Africa's production (Taylor, 2002).

2.6.2. Production of sorghum in Ethiopia

In Eastern Africa, more than 70% of sorghum is cultivated in the dry and hot lowlands where serious water deficit is the major production constraint. In Ethiopia, sorghum is grown as one of the major food cereals. Annually 1.3 million hectares of land is allotted for sorghum production and 1.7 million tons of grain is produced in the country. Sorghum is grown in different agro-ecological zones, but predominantly cultivated in dry areas that cover nearly 66% of the total area of the country (Gebisa et al., 2008).

Sorghum in Ethiopia is produced both in highland temperate mixed farming systems and mixed maize systems. As mentioned above, Ethiopia contains the majority of Sub-Saharan Africa (SSA's) highland temperate mixed zones. The maize mixed system covers 19% of cultivated area and provides livelihoods for 15% of the population in SSA (Wortmann et al., 2009). Contrary to regional trends, men are primarily responsible for sorghum production and marketing in Ethiopia. Women are primarily responsible for post-harvest handling. Sorghum is typically planted between April and July and harvested from October to December (Waddington et al., 2009).

Ethiopian sorghum yields exceeded both regional and world average yields in 2008. However, average yields still remain well below mean U.S. yields, for example, which average 4,300 kilograms per hectare. Therefore, although yields are above East African and world average yields, Waddington et al. (2009) suggest there is significant room for improvement. Expert panelist responses identified a smallholder sorghum yield gap in SSA of 1,640 kilograms per hectare in highland temperate mixed systems and 1,230 kilograms per hectare in mixed maize systems. The highest smallholder yield was 3,440 kilograms per hectare in the highlands and 2,930 in mixed maize areas. However, the regional average smallholder yields were only 1,800 and 1,130 kilograms per hectare for each farming system, respectively (Waddington et al. 2009). In 2008, Ethiopia harvested 1,533,537 hectares of sorghum. Closing the yield gap would therefore produce between 1,886,250 and 2,515,000 additional tonnes of sorghum.

Adugna and Tesso (2006) tested the potential of 14 sorghum hybrids in different environments in Ethiopia. They recommend four hybrid varieties (genotypes 1, 2, 3 and 5) with good stability and yield as suitable for drought-stressed areas of Ethiopia (Adugna and Tesso, 2006). However, Adugna (2007) notes that these improved varieties have not been well assimilated in Ethiopia. In addition to poor extension linkages limiting farmers' access to information about improved varieties, the lack of widespread dissemination may result from several other factors. Firstly, in a good season, the local cultivar is still higher yielding than the hybrid varieties. Secondly, the early maturing varieties, specifically, are more vulnerable to bird attack. And finally, the hybrid varieties have reduced biomass and therefore less value to farmers as animal feed (Adugna, 2007). Improved varieties could increase sorghum production in Ethiopia; however sorghum productivity faces additional constraints that limit this agronomic potential (Schneider and Leigh, 2010).

The tannin content of red sorghum grain is always higher than that of white sorghum grains. The high tannin content of red sorghum seeds inhibits the activity of some enzymes and therefore adversely influences protein digestibility and cellulose breakdown. Animal tests have proved that tannin inhibits protein absorption, decreases utilization of minerals and results in some decrease of growth. Feeding pigs with fodder containing 4.21% tannin decreased protein digestibility by 5.6%.

Amazing features of white sorghum (USDA, 2004):

- ✓ It has strong resistance to harsh environments such as dry weather and high temperature in comparison with other variety. It is usually grown as a low-level chemical treatment crop with limited use of pesticides.
- ✓ It has the potential to adapt itself to the given natural environment. It can be called "Nature-cared Crop" as it requires little artificial care such as irrigation and insect removal.
- ✓ It can be added to a variety of foods as it is almost taste and scent free and naturally white in color.
- ✓ The material advantages of other added ingredients such as taste are not harmed.

- ✓ Unlike wheat flour, it does not contain gluten. This makes it a suitable alternative food for people with wheat gluten allergies.

2.6.3. Nutritional composition of sorghum

Table 2.2 shows the composition of sorghum grain per 100g edible portion. Sorghum grain is first limiting in lysine, then in tryptophan. Much of the protein in sorghum is prolamine (39–73%) and methionine which are poorly digestible. As a result, maximum available protein in sorghum grain is usually 8–9%. Sorghum grain does not contain gluten and cannot be used for leavened products unless mixed with wheat. Decortication, parboiling, malting or steeping in alkali solutions and fermentation significantly improves the nutritional content of sorghum grain (USDA, 2004)..

Table 2.2 Nutritional composition of sorghum grain per 100g edible portion (USDA, 2004)

Proximate composition	g/100g	Minerals & vitamins	mg/100g	Essential Amino acids (AA)	mg/100g	Principal fatty acid (FA)	mg/100g
moisture	9.2	Calcium	28m	Tryptophan	124	Linoleic acid	1305
Protein	11.3	Iron	4.4	Lysine	220	Oleic acid	964
Fat	3.3	Phosphorus	287	Methionine	169	Palmitic acid	407
Energy	339Kcal	Vitamin A	0IU	Phenylalanine	546		
Carbohydrate	74.6	Thiamin	0.24	Threonine	346		
		Riboflavin	0.12	Valine	561		
		Niacin	2.9	Leucine	1491		
				isoleucine	433		

2.7. Chickpea (*Cicer arietinum* L.,)

2.7.1. Distribution of chickpea in the world

Chickpea (*Cicer arietinum* L.), also called garbanzo bean or Bengal gram, is an old world pulse and one of the seven Neolithic founder crops in the Fertile Crescent of the Near East (Lev-Yadun, 2000). Globally, chickpea is the third most important pulse crop in production, next to dry beans and field pea. During 2006-09, the global chickpea

production area was about 11.3 million ha, with production of 9.6 million metric tonnes (mmt) and average yield of 849 kg/ ha. India is the largest chickpea producing country with an average production of 6.38 million MT during 2006-09, accounting for 66% of global chickpea production. The other major chickpea producing countries include Pakistan, Turkey, Australia, Myanmar, Ethiopia, Iran, Mexico, Canada and USA. There are two distinct types of cultivated chickpea, Desi and Kabuli. Desi (*microsperma*) types have pink flowers, anthocyanin pigmentation on stems, and a colored and thick seed coat. The kabuli (*macrosperma*) types have white flowers, lack anthocyanin pigmentation on stem, white or beige-colored seeds with a ram's head shape, thin seed coat and smooth seed surface (Jukanti, 2010).

2.7.2. Production of chickpea in Ethiopia

In Ethiopia, the earliest finding of chickpea is reported in 1520 BC (Joshi *et al.*, 2001). Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent's production during 1994-2006. It is also the seventh largest producer worldwide and contributes about 2% to the total world chickpea production. Chickpea, locally known as *shimbra*, is one of the major pulse crops (including faba bean, field pea, haricot bean, lentil and grass pea) in Ethiopia and in terms of production it is the second most important legume crop after faba beans. It contributed about 16% of the total pulse production during 1999-2008. The total annual average (1999-2008) chickpea production is estimated at about 173 thousand tonnes. During the same period, chickpea was third after faba beans and field peas in terms of area coverage (Jones *et al.*, 2006).

Chickpea production using residual moisture at the end of August has become an integral part of the teff and wheat production systems in the vertisols of the Ethiopian highlands where it is rotated with the cereals for enhancement of soil fertility. Chickpea generates cash income and improves food and nutritional security for smallholder farmers. In the past the food preparations were mainly by way of roasting or boiling but now this has included the making of flour for preparation of sauce used in eating Injera, the most popular Ethiopian soft bread made from teff and other cereals.

While Desi chickpea has been widely grown in Ethiopia for household food needs and domestic markets, production of Kabuli types is a fairly recent practice. Lack of a viable seed supply system has affected the availability of improved Kabulu and Desi type chickpea to smallholder producers. Traditionally, the Debre Zeit Agricultural Research Centre (DZARC), under the Ethiopian Institute of Agricultural Research (EIAR), has provided chickpea breeder seed to the Ethiopian Seed Enterprise (ESE) which in turn multiplies it to produce pre-basic and basic or foundation seed in its own farms. The ESE then sells the foundation seed directly to contracted farmers for multiplication of certified seed that is sold to the Agricultural Extension Departments for distribution in the different regions (Kassie et al., 2009).

2.7.3. Nutritional composition of chickpea

Table 2.3 shows nutritional composition of chickpea seeds per 100g portion. It is well known that plant proteins are an alternative to proteins from animal sources for human nutrition. Legumes are recognized as the best source of vegetable protein legumes. However, in recent years, there has been an increasing interest in other legumes such as chickpea (*Cicer arietinum* L.). Pulses, including beans and chickpea are one of the most important crops in the world because of their nutritional quality. Due to their good balance of amino acid, high protein bioavailability and relatively low levels of anti-nutritional factors, chickpea seed have been considered a suitable source of dietary proteins.

Table 2.3 Nutritional composition of chickpea seeds per 100g portion (Goni, 2003)

Proximate values		Mineral values		Vitamin values	
	g/100g		mg/100g		/100g
Moisture	10.35	Calcium	105	Vitamin A	149IU
Protein	23.50	Iron	4.40	Vitamin C	1.8
Fat	2.20	Zinc	3	Thiamin	0.7
Carbohydrate	65	Phosphorus	366	riboflavin	0.2
Energy	395	Manganese	2.20	Niacin	2.9

They are rich sources of complex carbohydrates, protein, vitamins and mineral. Chickpea seed has a high protein digestibility, contains high levels of complex carbohydrates (low glycaemic index), is rich in vitamins and minerals and is relatively free from anti-nutritional factors (Goni and Valentine- G., 2003). Pulses have shown numerous health benefits, e.g. lower glycemic index for people with diabetes (Goni and Valentine-G., 2003), increased satiation and cancer prevention as well as protection against cardiovascular diseases due to their dietary fiber content. Chickpea seed is processed and cooked in a variety of forms depending upon traditional practices and taste preferences. Different domestic processing methods (decortications, soaking, sprouting, fermentation, boiling, roasting, parching frying, and steaming) was used to obtain a suitable texture for the consumer, improvement in the nutritional factors and increase the protein digestibility (Esmat et al, 2010).

2.8. Anti-nutritional Factors

An antinutrient is a substance occurring in the diet which acts antagonistically towards one or multiple nutrients, reducing bioavailability. This is usually done through complex formation which reduces nutrient absorption (Graham et al., 2000). The nutritional value of cereals and legumes may be adversely affected by the presence of anti-physiological or toxic substances such as trypsin and chemotrypsin inhibitors, phytates, lectins, polyphenols, flatulence causing agents, cyanogenic compounds, lathyrogens, esterogens, goiterogens, saponins, anti-vitamins and allergens. These substances reduce the nutritive value of foods by inhibiting digestibility and utilization of proteins. It is therefore necessary to eliminate these substances by processing and genetic manipulation. Processing plays an important role in improving nutritive value of pulses and by decreasing antinutritional factors up to a tolerable limit.

2.8.1. Tannins in chickpea

Tannins are polyphenol components prevalent in food legumes. Studies have shown that tannins interact with proteins, enzymes or nonenzymes, and form tannin-protein complexes, which decrease protein digestibility and protein solubility. This decrease in protein digestibility may be caused by either the inactivation of digestive enzyme or the

reduction of the susceptibility of the substrate proteins after forming the complex. Polyphenols are known to interact with proteins and cause either inactivation of enzyme such as trypsin and chymotrypsin or make protein insoluble. Polyphenols inhibit several enzymes including α -amylase, lipases, pectin esterases, cellulases and β -galactosidase. In addition to this, tannins reduce the bioavailability of vitamins and minerals (Chavan et al., 1986).

Chickpea seeds (whole seed) contain 78 to 272 mg tannins per 100 g seeds; while the cotyledons have only 16 to 38 mg per 100 g seeds. Tannins are mainly located in the seed coat. There is a considerable variation in seed coat color among the various chickpeas cultivars. The polyphenols in cultivars, which have darker testa color, inhibit the digestive enzyme activity more than cultivars with lighter testa color. These components impart astringent flavors, which are not always desirable. Some processing treatments such as dehulling and cooking considerably reduce the level of tannins in legumes. As such, chickpea seeds with light color are preferred for whole seed consumption (Chavan et al., 1986).

2.8.2. Tannins in sorghum

All sorghums contain phenolic compounds, including phenolic acids and flavonoids. Some contain in the layer under the seed coat condensed polyphenols called tannins but most cultivated sorghums do not contain any. These compounds can affect color, flavor and nutritional quality of the grain and products prepared from it. Tannins protect the grain against insects and birds (conferring a bitter, hit taste). The tannin content of seeds inhibits the activity of some enzymes and therefore adversely influences protein digestibility and cellulose breakdown. Animal tests have proved that tannin inhibits protein absorption, decreases utilization of minerals and results in some decrease of growth. Feeding pigs with fodder containing 4.21% tannin decreased protein digestibility by 5.6%. Before ripening the tannin content of grain is always higher than after ripening. The tannin content of dark grains is always higher than that of pale grains. Some white sorghum varieties have pigmentation in the pericarp and testa, due to phenolic compounds (Irén Léder, 2004).

2.8.3. Phytates in chickpea

Phytate, which is also known as inositol hexakisphosphate, is a phosphorus containing compound that binds with minerals and inhibits mineral absorption. The presence of phytate in foods has been associated with reduced mineral absorption due to the structure of phytate which has high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing non-availability for intestinal absorption (Walter et al., 2002). Phytates are generally found in food high in fiber especially in wheat bran, whole grains and legumes (Lori et al., 2001).

The major concern about the presence of phytate in the human diet is its negative effect on mineral uptake. Minerals of concern in this regard include zinc, iron, calcium, magnesium, manganese and copper (Lopez et al., 2002). The formation of insoluble mineral-phytate complexes at physiological pH values is regarded as the major reason for the poor mineral bioavailability, because these complexes are essentially non absorbable from the human gastrointestinal tract. Furthermore, the human small intestine has only a very limited capability to hydrolyse phytate due to the lack of endogenous phytate degrading enzymes and the limited microbial population in the upper part of the digestive tract. Solubility and stability of myo-inositol phosphate--mineral complexes have been found to decrease as the number of phosphate residues on the myo-inositol ring decreases.

Therefore, removal of phosphate residues from phytate results in a reduced impairment of intestinal uptake of essential dietary minerals (Sandberg et al., 1999). In isolated form only myo-inositol pentakisphosphate suppressed absorption of iron, zinc and calcium in humans, while myo-inositol tetrakis- and trisphosphates had no effect in the concentrations under investigation. In the presence of higher phosphorylated myo-inositol phosphates, however, myo-inositol tetrakis- and trisphosphates were shown to contribute to the negative effect of phytate on iron absorption. Because a strong negative correlation was found between zinc absorption and the sum of myo-inositol tris- through hexakisphosphate from cereal and legume meals, such a contribution is probably also true for zinc absorption (Sandberg, 1991).

Phytic acid binds trace elements and macro-elements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract are making dietary minerals unavailable for absorption and utilization by the body. It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis. Moreover, the phosphorus in phytate has been considered to be largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyse phytate in the small intestine. Phytate is also known to form complexes with proteins at both acidic and alkaline pH. This interaction may affect changes in protein structure that can decrease enzymatic activity, protein solubility and proteolytic digestibility. However, the significance of protein-phytate complexes in nutrition is still under scrutiny. Strong evidence exists that phytate-protein interactions negatively affect protein digestibility *in vitro* and the extent of this effect depends on the protein source (Sandberg, 1991)..

2.8.4. Phytic acid in sorghum

Sorghum bran contains the highest levels of phytates. Forty to fifty percent of phytate and total phosphorus can be removed by abrasive dehulling (Irén Léder, 2004). It is usually located in the outer layer of cereals where the fiber is primarily concentrated. Phytic acid is highest in whole-grain flours and can be decreased considerably by removing its hull (Hurrell, 2003). Phytic acid form complex compounds with cations such as zinc, iron, magnesium and calcium at physiological pH. It is either insoluble or difficult to hydrolyze during digestion. Phytic acid inhibits minerals (such as iron, zinc) absorption by humans and other monogastric animals in a dose-dependent manner. When the molar ratio of phytate: iron and phytate: zinc is above 0.15 and 15, respectively, phytate inhibits absorption of minerals (Allen and Ahluwalia, 1997; Dicko, 2005 and Melaku, 2005). The antinutritional effect of phytic acid is primarily related to its strong chelating ability with minerals and proteins. It is intern associated with its six reactive phosphate groups. Multivalent cations are particularly susceptible and form insoluble, indigestible complexes. Phytates also affect enzyme activity with a negative effect for key digestive enzymes including amylase, pepsin and trypsin (Dicko, 2005).

2.9. Minerals

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 and polyphenols (Idris, 2005; 2007). Bioavailability is the degree to which the amount of an ingested nutrient is absorbed and available to the body (WFP, 2006). Mineral bioavailability is affected by different factors including antinutritional factors, chemical form of the mineral, and interaction with other minerals, presence or absence of certain vitamins and the type of food from which the mineral is obtained (WFP, 2006). An adequate mineral absorption is important especially for infants, children, elderly people and people in clinical situation (Idris et al., 2005). It is evident that the nutritional importance of a given food stuff depends not only on nutrient composition of raw food stuff but also on the amount utilized (Idris et al., 2007).

2.9.1. Zinc

Zinc deficiency is a public health problem, and is associated with poor growth, decreased immune function, increased susceptibility to and severity of infections, adverse outcomes of pregnancy, and neurobehavioral abnormalities (Sandberg, 2002; Melaku et al., 2005). Zinc is an essential trace mineral that is a component of over 200 enzymes and is known to be necessary for normal collagen synthesis and mineralization of bones, and is involved in vital processes such as mitosis, synthesis of DNA and protein, and gene expression and activation (Walingo, 2009). Deficiency of Zn is highly prevalent in developing countries, but also in vulnerable groups with high requirements in industrialized countries, such as women of fertile age, infants and adolescents (Sandberg, 2002). Zinc deficiency is presumed to be the underlying cause of stunting and delayed sexual maturation. 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 nutritional status influences the absorption, transport and utilization of vitamin A (Adeyeye et al., 2000).

2.9.2. Iron

Iron is a micronutrient that is most often deficient in developing countries, with children and women of reproductive age especially at risk of such deficiencies (Melaku et al.,

2005). More than half (54 percent) of Ethiopian children 6-59 months old are anaemic, with 21 percent mildly anaemic, 28 percent moderately anaemic, and 4 percent severely anaemic (EDHS, 2005). Low content and bioavailability of iron in the typical cereal-based diet is a major cause of iron deficiency (Sandberg, 2002). This is due to the high content of antinutrients in most cereals and other plant source staple foods and inadequate intake of animal foods in the diet (Sandberg, 2002; Melaku et al., 2005).

2.9.3. Calcium

Calcium is the most abundant mineral element in the body. Calcium (small proportions) regulates critical functions including nerve impulses, muscle contractions and the activities of enzymes and (more than 99%) is located in the bones, plays an important role for structure and strength of bones. Sufficient calcium intake is essential for obtaining optimal peak bone mass in youth and for minimizing bone loss later in life (Gurr, 1999). Up to about a third of calcium from food is absorbed, the remainder being excreted in the faeces. The proportion of calcium absorbed from food depends on how the calcium is chemically bound in the food and the presence of many substances also present in the food, which may either enhance or inhibit absorption. The efficiency of calcium absorption decreases as the amount ingested increases (Gurr, 1999).

2.9.4. Phosphorus

Phosphorus is a major component of the body's structural calcium-phosphate complex-hydroxyapatite in bone and fluorapatite in dental enamel. Phosphate is involved in the structure of the genetic materials DNA and RNA, and other essential roles of this mineral include energy transfer and storage, structure of phospholipids in cell membranes and maintenance of acid base balance. Most dietary phosphorus is in the form of phosphates. Phosphorus deficiency can result from excessive use of antacid that contain aluminum phosphate in the intestine.

Deficiency of phosphorus is common in malnourished children and severe hypophosphatemia is associated with increased mortality in kwashiorkor. Phosphorus deficiency is also likely to cause rickets-like bone changes in malnourished children.

Phosphorous is likely to be a limiting nutrient in treatment of children. Absorption of dietary phosphorus is high (55-70%), relatively independent of dietary composition, and does not appear to be up-regulated at low intakes. Dairy products, meat, poultry, eggs, fish, nuts, and legumes are generally good sources of highly available phosphorus. However, the main form of phosphorus from plant material is phytate which is resistant to digestion unless enzymatically degraded by phytase. Thus, phosphorus from phytate is only absorbed to a minor degree under normal conditions and the phytate fraction of phosphorous should therefore be discounted from the calculations of the total phosphorous requirements (Golden, 2009).

Chapter Three

3. Materials and Methods

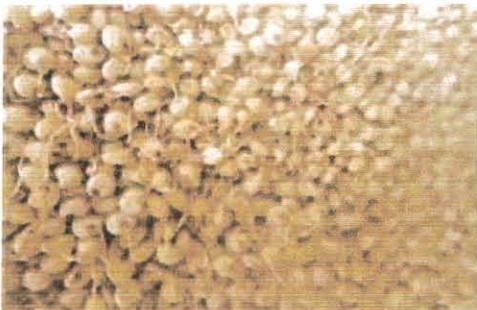
3.1. Selection of sampling site and sampling method

3.1.1. Sampling site

Law tannin sorghum (*Sorghum bicolor* L. Moench) variety (“Teshale”) was collected from Melkassa Agricultural Research Centre (Meiso) and chickpea (*Cicer arietinum* L.) variety (“Harbu”) was obtained from Debre Zeit Agricultural Research Centre (DZARC) and stored in clear sterile polyethylene bags and kept at room temperature until use for three weeks. Melkassa and Debre Zeit research sites were chosen for this study for reasons of accessibility and potential areas of sorghum and chickpea varieties produced and these research sites are also working mainly in cereals and legumes, respectively.



Kabuli chickpea variety (“Harbu”)



Germinated sorghum (“Teshale”)



Sorghum (“Teshale”) variety

Figure 3.1 sorghum and chickpea samples variety used in the study.

3.1.2. Sampling method

Law tannin sorghum variety (“Teshale”) and kabuli chickpea variety (“Harbu”) were used throughout this study (Figure 3.1). The sampling technique used was convenience (Availability) sampling method; sorghum from sorghum extension department under Melkassa research site (“Meisso”) and chickpea from chickpea extension department in Debre Zeit research center. In addition, samples were checked not damaged during harvest and not attacked by pests. About 4.0 kilograms of sorghum and 1.5 kilograms of chickpea samples were collected from each variety.

3.2. Sample preparation

The sorghum and chickpea samples collected for this study were manually cleaned to remove stones, dust and light materials, stalks, and broken undersized and immature grains. Cleaning was done by winnowing and hand sorting. After cleaning sorghum grains were divided into two portions:

- ✓ One part (ungerminated); used as control was dried, milled, sieved/kneaded and ready for analysis.
- ✓ The second part; soaked for 22 hours, germinated for 2 days (Shimelis, 2009), dried, milled, sieved and ready for fermentation.

The chickpea was blanched for 20 minutes, dehulled manually, dried by drying oven, milled, sieved and ready for blending proportion and fermentation. Ungerminated chickpea was used throughout this study to blend with germinated and ungerminated sorghum. Raw sorghum (100%) and raw sorghum/chickpea blend (70:30) were used for comparison with the treated samples. For drying purpose, drying oven (Memmert, Germany) was adjusted at 55 °C for about 24 hours. The dried samples (4000g sorghum and 1500g chickpea) were milled into flour using Tecator Cyclotec1093 sample miller (Sweden) and passed through a 1 mm aperture size laboratory test sieve (Endecotts Ltd., London England). Sieved samples were then packed in airtight polyethylene plastic bags until further analysis (Figure 3.2).

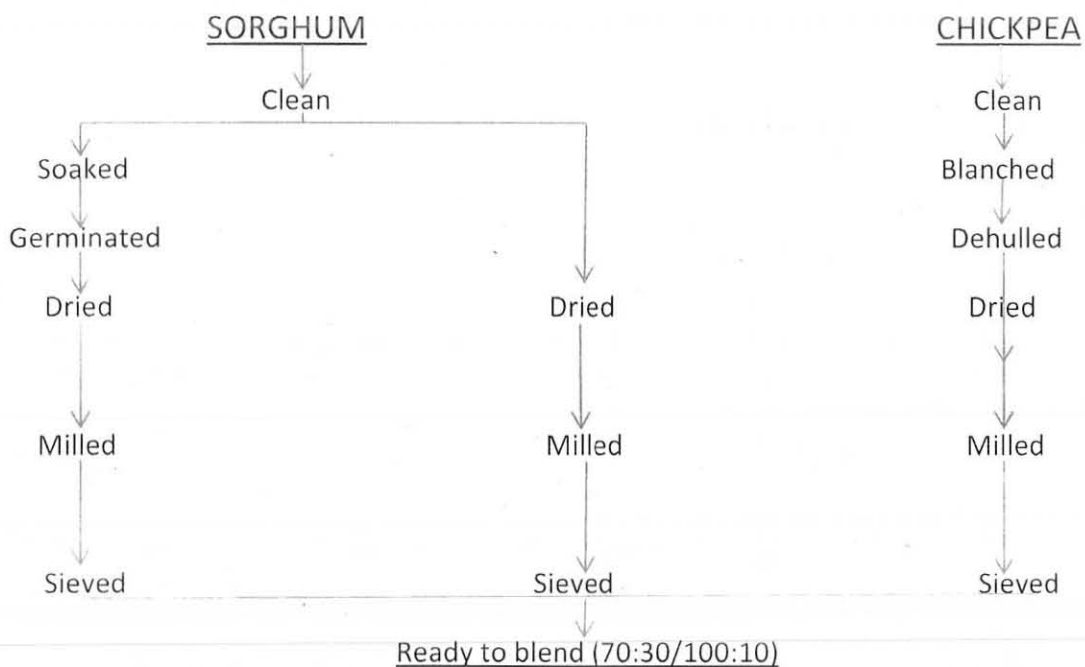


Figure 3.2 Flow chart of sample preparation

3.3. Description of the processing conditions

3.3.1. Germination

Sorghum grains were washed and soaked for 22 hours then washed twice using deionized water. The soaked and washed sorghum grains were again divided into two portions. The first portion was germinated for 22 hours (Shimelis, 2007) and the second portion remained ungerminated. During germination seeds were covered with wet clean cloth and placed in a clean bamboo-made basket. Then left in ambient conditions and watered 2-3 times a day to enhance the germination process.

3.3.2. Fermentation

Blended sorghum and chickpea flour with the blending ratio of sorghum to chickpea: 70:30 and 100:00 were used and prepared in de-ionized water at a concentration of 1:3 dilutions (w/v) in plastic containers. The prepared slurry of sorghum and chickpea were allowed to ferment in two ways: -

- ✓ Naturally with only the microorganisms borne on or inside the seeds (endogenous microflora on the seeds) and
- ✓ Using germinated sorghum flour at room temperature (25 ± 2 °C) for 72 hrs.

During the fermentation process samples were withdrawn within 24 hrs interval, and pH, titratable acidity and microbial analysis were performed to determine appropriate physico-chemical and microbiological properties of complementary foods. After pH and total bacterial count were performed, it was approved 48 hrs fermentation was optimal for the lower limit of recommended microbial load of complementary food. So, 48hrs fermented samples were transferred to aluminium dishes and dried in a freeze drier for further analysis. Dried samples were milled with a miller (IKA-Werke, M 20) to pass a 1 mm sieve and stored for analysis. Dried samples were analyzed for pH, titratable acidity, bulk density, microbial, proximate analysis, mineral (Zn, Fe, Ca and P) tannins, phytate, viscosity and sensory characteristics.

3.4. Experimental design for preparation of porridge

At a low flour content (5–15g dry matter/100 ml), the gruel has a free-flowing consistency and is easy to swallow but its energy density (20–40 kcal/100 g) is lower than the minimum value of 84 kcal/100 g of gruel recommended for children aged 9–23 months fed at a rate of 2 meals/day in addition to average breast milk intake (Dewey & Brown, 2003), so that 17% and 25% dry matter (DM) was selected for preparation of porridge. Raw, germinated and fermented sorghum/chickpea flours were mixed with water in the proportion of sample to water (17%: 83%) and (25%: 75%). Then, porridges were prepared by mixing flour and water in a 200ml glass beaker at 92 °C for 15 minutes.

Eight porridges were prepared at dry matter (17% and 25%) contents and with 70:30 and 100:100 (sorghum: chickpea) blending proportions (Figure 3.3). This blending proportion, which corresponds to the energy and macronutrient requirements of young children, was used based on the material balance method to target 18% protein and 59% carbohydrate in the complementary food formulation (Amankwah, 2009). The dry matter

content of porridges was determined by oven-drying at 105 °C to constant weight (for 6 hours).

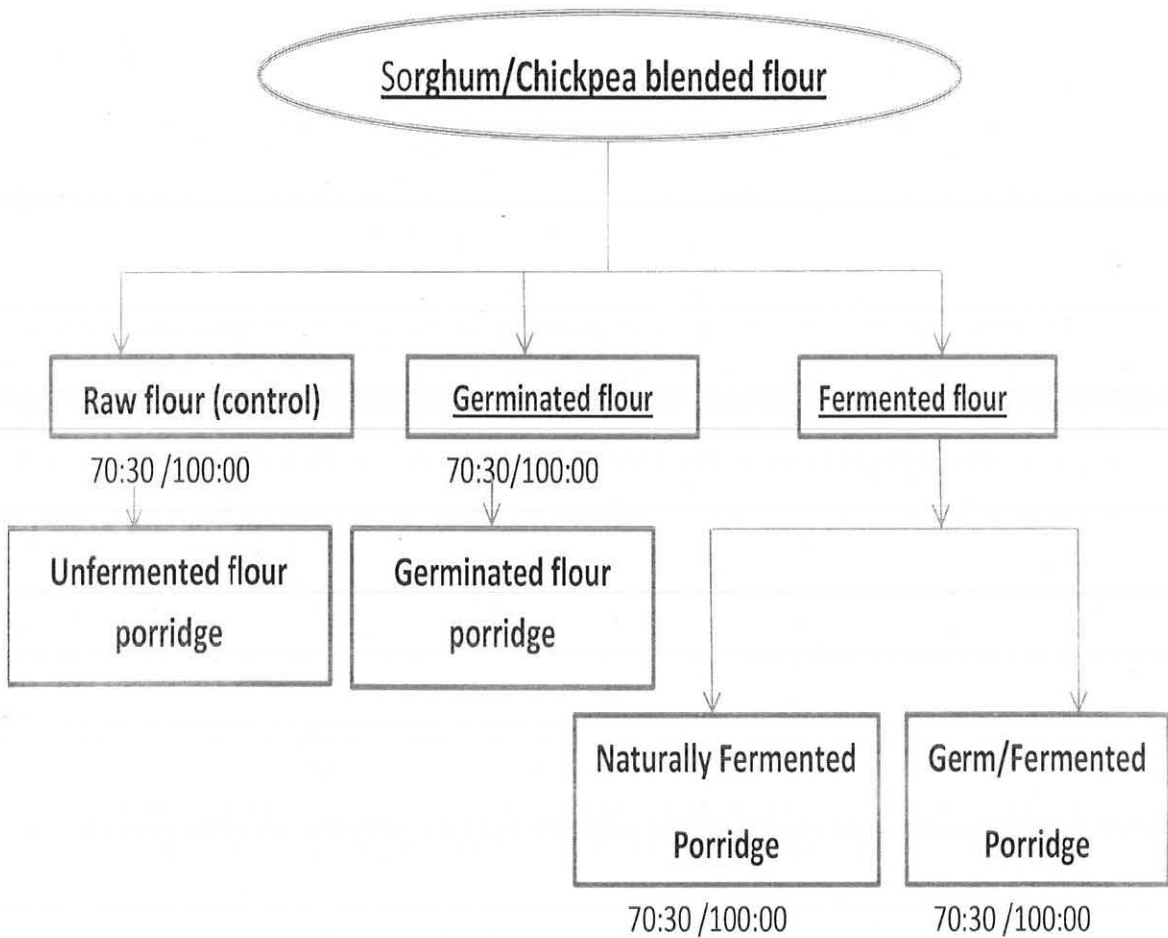


Figure 3.3 Experimental design showing the germination and fermentation processes to prepare porridge.

3.5. Laboratory analysis

3.5.1. PH and titratable acidity determination

The pH and titratable acidity of the samples were determined according to the method of Pearson (1971). 10 g of the sample was added to 100 ml of distilled water and stirred for 30 min. The pH of the slurry was determined by dipping the electrode of the pH meter

(Hanna Instrument- pH 301) in the mixture and the result was recorded. Triplicate determinations were made in all cases. The pH meter was calibrated using pH 4.0 and 7.0 buffers. Titratable acidity expressed as percentage of lactic acid, was determined by titrating 10 ml of the homogenate samples used for pH determination against 0.1 N NaOH using phenolphthalein indicator. The volume of 0.1 N NaOH used for titration of the sample was recorded.

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

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 C₃H₆O₅; W=Weight in gram of sample in the mixture (Pearson, 1971).

3.5.2. Methods for determination of microorganisms

Total plate counts and lactic acid bacteria counts were isolated within 24 hours interval from samples taken during the fermentation step (from 0 to 72 h) after serial decimal dilutions in 9 (w/v) sodium chloride solutions. Total plate count and lactic acid bacteria (LAB) were enumerated using Plate Count Agar (PCA, Difco, France) and de Man, Rogosa and Sharp (MRS, Difco, France). MRS plates were incubated at 30°C for 48 h, PCA plates at 37 °C for 48h. The plates containing 30 to 300 colonies were counted using a colony counter. Results are means of duplicate enumerations of microflora in samples of each experiment.

3.5.3. Bulk density

Bulk density was determined by using the method described by Narayana and Narasinga (1984). Ten grams of each sample was weighed (W1) into a 25 ml graduated measuring cylinder. The sample was gently tapped to eliminate spaces between the flour samples and reweighed (W2). The study was conducted in duplicate.

$$\text{Bulk density} \left(\frac{g}{ml} \right) = \frac{W1 - W2}{\text{Volume of sample before tapping}}$$

Where: W1= weight of sample before tapping

W2= weight of sample after tapping

3.6. Proximate Analysis

3.6.1. Determination of moisture content

The dishes used for the moisture determination were dried at 130 °C for 1 hr in Memmert drying oven of model 40050 and placed in desiccators for about 30 min. The mass of each dishes was measured (M1) and about 5 g of the sample was weighed in to each of the dishes (M2). The sample was then mixed thoroughly and dried at 100 °C for 6 hr. After drying is completed, the mass was measured (M3). The moisture content was calculated from the equation:

$$\text{Moisture content } \left(\% \frac{w}{w} \right) = \frac{M2 - M3}{M2 - M1} * 100$$

Where: M1=mass of the dish, M2=mass of the dish and the sample before drying, and M3=mass of the dish and the sample after drying (AOAC 2000, 925.05).

3.6.2. Determination of crude protein content

Protein content was determined according to AOAC (2000) using the official method 979.09. About 0.5g samples were taken in a digestion flask and 6ml of concentrated sulfuric acid was added and left overnight. Then 3.5ml of 30% hydrogen peroxide and 3g of the catalyst mixture (75g of copper sulfate and 0.7g of potassium sulfate) were added in to digestion flask. The mixture was then exposed to a temperature of about 370 °c for four hours in order to allow digestion. Then distillation was took place by adding 25ml of 40% sodium hydroxide to the digestion flask and using 25ml of 4% boric acid solution and three drops of methyl red solution as indicator into a 250ml conical flask. The distillation was continued until a total volume of 150-200ml is collected in conical flask. Finally, the distilled solution was titrated with standardized 0.1N hydrochloric acid to a pink color and the amount of hydrochloric acid was recorded. The crude protein content was estimated using the formula:

$$\text{Crude nitrogen content } (\%) = \frac{(A - B) * N * 14.007}{W} * 100$$

Where: A= volume (ml) of hydrochloric acid used in titration for the test sample

B = volume (ml) of hydrochloric acid used in titration of the blank

N = normality of hydrochloric acid used (0.1N)

14.007= Equivalent weight of nitrogen

W= weight in gram of a test sample

The protein content was calculated from the following equation:

$$\text{Crude protein (\%)} = \% \text{Total nitrogen} \times 6.25$$

3.6.3. Determination of crude fiber content

Crude fiber content was determined according to AOAC (2000) using official method 962.09. About 1.5g weighed sample was placed into a 600ml beaker and about 200ml of 1.25% H₂SO₄ was added and boiled for 30 minutes. Recording was taken place by placing a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20ml of 28% KOH was added and again boiled gently for a further 30 minutes. Subsequently washing was conducted with 1% sulfuric acid and NaOH solution and filtering was followed. After filtering the sample was dried in an electric oven at 130 °C for 2 hours and cooled for 30 minutes in the desiccators and weighed. Then it was ashed in a muffle furnace ashing for 30 minutes at 550 °C and cooled in the desiccators and then re-weighed. Finally, the crude fiber content was calculated by using the following formula:

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2}{W_3} * 100$$

Where: W₁=weight of crucible and sample after drying

W₂=weight of crucible and sample after ashing,

W₃=weight of sample

3.6.4. Determination of total ash content

Total ash content was determined according to AOAC (2000) using the official method 923.03. The porcelain dish used for the analysis was cleaned by drying oven at 105 °C for 30 minutes and cooled in desiccators for 30 minutes was measured using analytical balance (M1). About 2.5g of the sample was weighed in to porcelain dish (M2). The

dishes and their content 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 charred samples were placed inside the muffle furnace (Carbolite CSF 1200) at about 550 °C for six hours until free from carbon and the residues appear whitish color. The sample was then removed from the furnace and if the ashing was incomplete (traces of carbon are still evident) it was moistened by the few drops of de-ionized water and conc. HNO₃ added, dried and return to the furnace and re-ash at 550 °C until white ash color was obtained. It was removed from the furnace and cooled in the desiccators and finally the mass was weighed (M₃). The amount of total ash was calculated by using the following formula:

$$\text{Total ash (\%)} = \frac{W_3 - W_1}{W_2 - W_1} * 100$$

Where: M₁=Weight of the dish

M₂= Weight of fresh sample and dish

M₃ = Weight of ash and dish

3.6.5. Determination of crude fat content

The crude fat content was determined according to AOAC (2000) using official method 4.5.01. The cleaned extraction flasks were dried by drying oven at 105 °c for 30 minutes and then cooled for 30 minutes in desiccators and weighed. A clean, dried extraction thimble containing about 2g of dried sample and covered with fat free cotton at the bottom and top was placed into Soxhlet (Shanghai Qianjian Instrument CO., LTD) extraction chamber. Cooling water was switched on and a 50ml of Diethyl Ether was added to the extraction flask through the condenser. The extraction was conducted for about four hours. Finally, extraction flasks were dried in oven at 105 °C for 30 minutes and cooled in the desiccators for 30 minutes and weighed. The crude fat content was determined as follows:

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

Where: W2 = Weight in gram of extraction flask and dried crude fat

W1 =Weight in gram of extraction flask

W=Weight in gram of test sample

3.6.6. Determination of total carbohydrate

Total carbohydrate content was calculated by difference using the formula as follows:

Carbohydrate (%) = 100 – (% crude protein + % crude fiber + % total ash + % crude fat)

3.6.7. Determination of total energy

Total energy content was obtained using Atwater conversion factors 4, 9 and 4 for each gram of crude protein, crude fat and carbohydrate and expressed in calories, respectively (Guyot *et al.*, 2007).

$$\text{Total energy} \left(\frac{\text{Kca}}{100\text{g}} \right) = (9 * \% \text{Fat}) + (4 * \% \text{Protein}) + (4 * \% \text{Carbohydrate})$$

3.7. Mineral analysis

3.7.1. Calcium, iron and zinc determination

Calcium, iron and zinc were determined according to the standard method of AOAC (2000) using an Atomic Absorption Spectrophotometer (Varian SAA-20 Plus). Ashing of the samples were followed by digestion and absorption. Ashing: carefully washed silica dishes were dried in oven at 105 °C for 15 minutes and cooled in desiccators about 30 minutes at room temperature and weighed. About 1.00g of sample was transferred into each dish and placed on a hot plate under a fume-hood in slowly increasing temperature until smoking ceases. The charred sample was placed in a muffle furnace at about 550 °C until free from carbon and the residues appear white in color. The samples were then removed from the muffle furnace and if the ashing was incomplete it was further moistened by a few drops of de-ionized water and concentrated nitric acid and dried on a hot plate and re-ashed in the furnace at 550 °C until white ash color was obtained.

Digestion: the ash was digested with 5ml of 6N HCl to wet it completely and carefully dried on a low temperature hot plate. Seven ml of 3N HCl was added on each dish and heated on a hot plate until the solution just boils. Then the solution was cooled to room temperature and filtered through a filter paper (Whatman) into 50ml volumetric flask retaining as much of the solids as possible in the dish. Again 5ml 3N HCl was added to the dish to dissolve the residue and it was heated until the solution just boiled. Then the solution was cooled and filtered in to the same 50ml volumetric flask. The dish was then washed with water and filtered in to the volumetric flask. Since calcium is to be determined 2.5ml of 10% Lanthanum chloride solution was added in 50ml volumetric flask that contained the filtrate. Finally, the flask was filled with freshly de-ionized water up to the mark. Blank was also prepared in parallel with the sample using the same amount of reagents that were used for the sample through all steps.

Standard solutions: Five series of working standard solutions for Ca, Fe and Zn were prepared from 1000ppm metals stock solutions with de-ionized water containing 2.4ml 3N HCl and 0.5ml Lanthanum chloride in 10ml volumetric flask used to make the sample and standard matrix similar. The stock standard solutions of minerals (zinc, calcium and iron) were diluted with 0.3N HCl to concentrations that fall within the working range (0.5, 1.5, 2.0, 2.5 and 3.0 (µg/ml) for calcium analysis; 2, 6, 10 and 12 (µg/ml) for iron analysis; 0.5, 1.0, 1.5 and 2.0 (µg/ml) for zinc analysis.

The apparatus were set according to the instructions and a calibration curve was prepared by plotting the absorption values against the metal concentration in (µg/ml). The Fe, Zn and Ca content in the sample was determined using Atomic Absorption Spectrophotometer at 248.3nm, 213.9nm and 422.7nm wavelengths, respectively. Three reading were taken from the graph for each metal which depicted the metal concentrations that correspond to the absorption values of the samples and the blank. The metal contents were calculated by using the following formula:

$$\text{Metal contents } \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{(A - B) * V}{10 * W}$$

Where: W=Weight of the sample in gram

A=Concentration ($\mu\text{g/ml}$) of sample solution V = Volume of the extract (50ml)
B=Concentration ($\mu\text{g/ml}$) of blank solution

3.7.2. Phosphorus determination

The sample solutions prepared for mineral determination was used for phosphorous determination. About 1ml of the clear extract was diluted into 50ml with de-ionized water in a 100ml volumetric flask. Five ml of the sample dilution was added into test tubes. A 0.5ml of molybdate and a 0.2ml aminonaphtholsulphonic acid were added into the test tubes (sample solution) and mixed thoroughly step by step. Again 0.2ml aminonaphtholsulphonic acid was added into the test tubes repeatedly until the solution becomes clear and then the solution was allowed to stand for 10 minutes. Standard and blank solutions were prepared as above but 5ml of working standard and 5ml of de-ionized water in place of the sample dilution were used, respectively. Six series of working standard phosphorus solutions (0, 0.2, 0.4, 0.6, 0.8 and 1.0 $\mu\text{g/ml}$) were prepared by dissolving 438.8mg of KHP_2O_4 in some water in a 100ml volumetric flask and added 1ml of concentrated H_2SO_4 and dilute to the mark with de-ionized water. After setting the instrument operation process, the absorbance (A) of the sample solution was measured at 660nm against distilled water using UV-VIS spectrometer. The standard and sample blank solution analysis was carried out simultaneously with sample solution. Calibration curve (concentration versus absorbance) prepared and used to measure the amount of phosphorus in the test sample as follows:

$$\text{Phosphorus} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{(A - B) * \text{dilution factor} * \text{extracted volume}}{\text{Slope} * \text{Weight of sample} * 1000} * 100$$

Where: A= absorbance of sample, B=absorbance of blank, Slope= obtained from the calibration

3.7.3. Determination of phytate and non-phytate phosphorus

Phytate and phosphorous were determined by the above methods. Phytate phosphorus was calculated with the following formula (Khetarpaul and Sharma, 1997).

$$\text{Phytate phosphorous (mg/100g)} = (A \times 28.18) / 100$$

Where: A = phytate content (mg/100g)

Non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus.

3.8. Analysis of antinutritional factors

3.8.1. Determination of phytate

Phytate content was determined using method described by Latta and Eskin (1980) and later modified by Vaintraub and Lapteva (1988). About 0.1g of dried sample was extracted with 10ml 0.2N HCl in a mechanical shaker for one hour at an ambient temperature and centrifuged at 3000rpm for 30 minutes. About 3ml of the clear supernatant (sample solution) was transferred into test tube for phytate estimation. About 2ml of wade reagent (0.03% solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.3% of sulfosalicylic acid in water) was added to sample solution and mixed with a vortex (Thermolyne mixer) for 5 seconds and centrifuged. Standard blank solution was prepared as above but 3ml of standard in place of the sample dilution was used.

Six series of working standard phytic acid solutions (0, 4.5, 9.0, 18, 27 and 36 $\mu\text{g}/\text{ml}$) were prepared by dissolving 0.1814g of phytic acid in 100ml of 0.2N HCl. After setting the instrument operation process, the absorbance (A) of the sample solution was measured at 500nm against de ionized water using UV-VIS spectrometer (Model CECIC, CE1021). The standard and sample blank solution analysis was carried out simultaneously with sample solution. Calibration curve (concentration versus absorbance) prepared and used to measure the amount of phytic acid in the test sample as follows:

$$\text{Phytic acid} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{\{(A - B) - \text{Intercept}\}}{(\text{Slope} * \text{Weight of sample})} * (10/3)$$

Where: A = absorbance of sample

B = absorbance of blank

Slope and Intercept = obtained from the calibration curve

3.8.2. Phytate mineral molar ratio calculation:

Phytate calcium molar ratio was obtained (mg of phytate/molecular weight of phytate divided by mg of calcium/molecular weight of calcium).

Phytate iron molar ratio was obtained (mg of phytate/molecular weight of phytate: mg of iron/molecular weight of iron).

Phytate zinc molar ratio was obtained (mg of phytate/molecular weight of phytate: mg of zinc /molecular weight of zinc).

Phytate x calcium/zinc millimolar ratio was obtained (mg of phytate/molecular weight of phytate) (mg of calcium/molecular weight of calcium)/ (mg of zinc/molecular weight of zinc) divided by 100.

3.8.3. Determination of condensed tannin

Tannin content was determined using the method of Burns (1971) as modified by Maxson and Rooney (1972). About 1g weighed sample was transferred into a screw cap test tube and 10ml of 1% HCl in methanol was added to extract and then the test tube was placed on mechanical shaker for 24 hours at room temperature. Then the sample solution was centrifuged (Adam smith) at 1000rpm for five minutes. About 1ml of supernatant was taken from sample solution and mixed (Vortex mixer) with 5ml of vanillin-HCl reagent (mixture of 4% vanillin in methanol and 8%HCl in methanol) in another test tube and then the mixture was left for 20 minutes to complete the reaction. Standard and blank solutions were prepared as above but (0.2-1.0) ml of stock solution and empty test tube were used in place of the sample solution, respectively.

Preparation of the standard curve: D-catechin was used as standard value for condensed tannin determination (tannin in mg D-Catechin/g of sample). Six series of stock solutions (0, 0.2, 0.4, 0.6, 0.8 and 1.µg/ml) were prepared by dissolving 40mg of D- catechin in 1000ml of 1% HCl in methanol. Then the volume of each test tube was adjusted to 1ml with 1% HCl in methanol and 5ml of vanillin-HCl reagent was added into each test tube and the test tube was kept for 20 minutes to complete the reaction. Finally, the

absorbance of sample solutions and the standard solution were measured at 500nm using UV-VIS spectrophotometer (model CE1021) and the standard curve was made from absorbance versus concentration. The slope and intercept were used for calculation. The condensed tannin content in the sample was calculated using formula:

$$\text{Condensed tannin } \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{(A-B) - \text{Intercept}}{\text{Slope} * W}$$

Where: A = Sample absorbance, d = Density of solution (0.791g/ml)

B = Blank absorbance, W = Weight of sample in gram

3.9. Viscosity determination

The porridges were prepared by mixing raw, germinated and fermented sorghum/chickpea flour and water in a 200ml glass beaker at a concentration of 17% and 25% dilutions (w/v). The mixture of water and flour were cooked at 92 °C for 15 minutes. The porridges were placed in a water bath maintained at 45°C (heating temperature) and its viscosity was measured using a Brookfield Viscometer (Model DVII Rheometer V2.0 RV; Middleboro, Massachusetts, USA) at Hawassa University. The cooked porridges were poured into the viscometer beaker, cooled to 45 °C and viscosity was measured in centipoises (cP), using spindle number 7 at a shear rate of 50 revolution per minute (RPM). After 10 minutes of revolution, the average of the maximum and minimum viscosity reading was recorded according to the speed.

3.10. Sensory evaluation of porridges

The sensory evaluation was carried out for consumer acceptance to evaluate the attributes such as color, aroma, taste, texture and overall acceptability of the porridge, prepared from raw (control), germinated and fermented sorghum/chickpea blend. Twenty two semi-trained panelists comprising of first year M.Sc. students from AAU Center for Food Science and Nutrition were randomly selected to perform the evaluation. Nine-point hedonic category scales was used to rate the attributes ranging from like extremely to dislike extremely as used by (Mepba *et al.*, 2007). Samples were evaluated on a desk

placed an open well illuminated room, which provided a quiet and comfortable environment.

In the sensory evaluation session, the porridge samples were prepared in the laboratory of Center for Food Science and Nutrition of Addis Ababa University, in the same condition that mothers used to prepare porridges at home. The panelists were seated individually in order to avoid dependency of one panel on the other. The coded samples were presented in random to each panelist on identical white transparent plastic tray at ambient temperature. Panelists were asked to indicate their degree of liking for each attributes of the coded samples by choosing the appropriate category. Panelists were provided with palatable water to rinse their mouth before and after evaluating each sample in order to eliminate bias between evaluations.

3.11. Design and Data Analysis

The Completely Randomized Design (CRD) was used in evaluation. The effect of fermentation and germination on the complementary blend was evaluated by comparing them to a control. The controls were formulated from 100% ungerminated sorghum (US) flour and 70% ungerminated sorghum flour with 30% chickpea (USC) blended flour. Data were submitted to analysis of variance (ANOVA) and subjected to SPSS package, version 20. Duncan's multiple range tests was used to separate means. Significance was accepted at the probability $P \leq 0.05$. Results were reported \pm standard deviation (SD) of duplicate and triplicate treatment and were averaged.

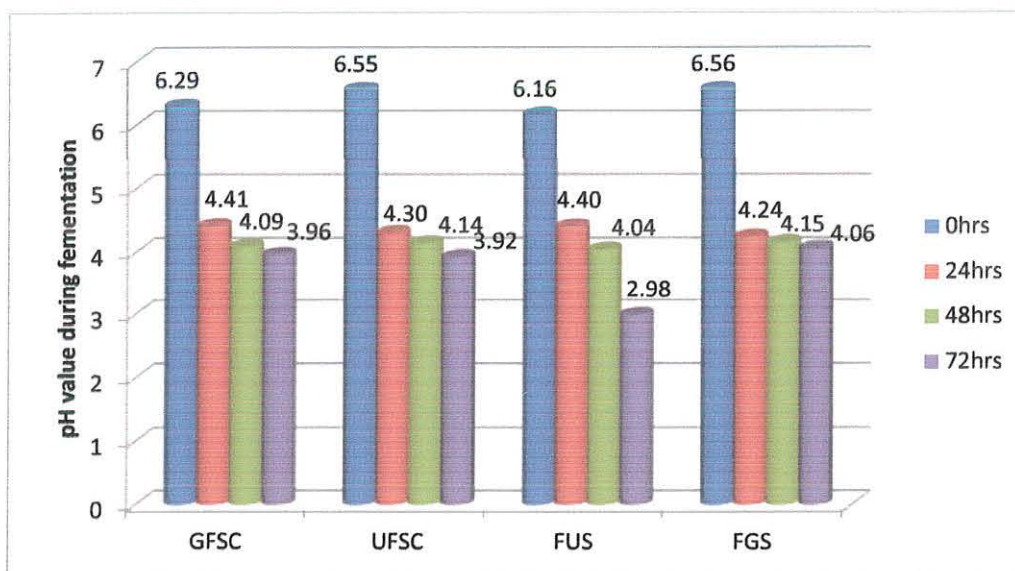
Chapter Four

4. Results and Discussions

4.1. Changes in pH during fermentation

The changes in pH during fermentation of sorghum/chickpea blend were illustrated in Figure 4.1. At the beginning of fermentation pH value of ungerminated sorghum (FUS), germinated/fermented sorghum (FGS), ungerminated/fermented sorghum/chickpea (UFSC) and germinated/fermented sorghum/chickpea (GFSC) were 6.56, 6.160, 6.55 and 6.29, respectively.

Figure 4.1 Changes in PH of sorghum/chickpea mixture during fermentation periods



Reported values are the mean \pm Standard Error SE (n=3).

FUS: - fermented/ungerminated

FGS: - fermented/germinated sorghum,

UFSC: - fermented/ungerminated sorghum/chickpea

GFSC: - fermented/germinated sorghum/chickpea.

During fermentation step (from 0 to 24h), acidification increased and resulted in a pH drop from an initial value of around 6.56 to a final value about 4.3 and followed by a

phase of slow acidification (from 24hrs to 48 hrs), that resulted in a further decrease in pH to about 4.04. Except in the fermentation of 100% germinated sorghum (GS), pH drop of all other combination of fermentation followed a similar and classical pattern as shown in (Figure 4.1). After 72 hours of fermentation the pH of most of the samples flour decreased steadily during fermentation and ranged between 2.98 (lowest value for germinated sorghum) and 4.09 (highest value for un-germinated sorghum).

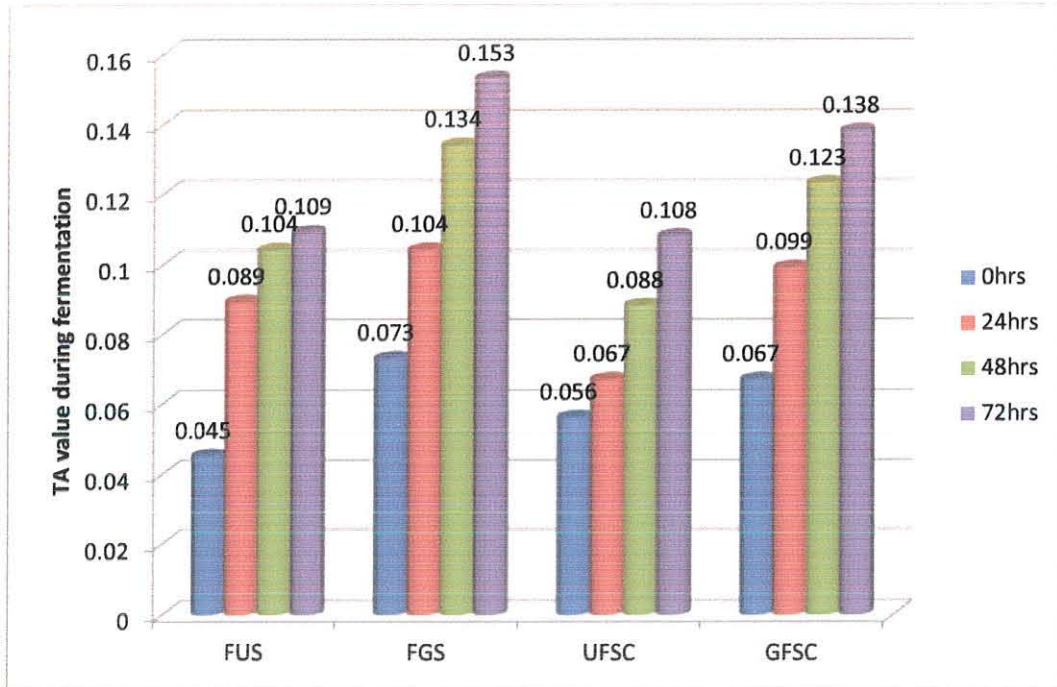
The effect of fermentation on pH was significantly influenced by the presence of germinated sorghum that was mixed during fermentation. These results agreed with Firibu (2012) who reported that when low or no malt was added to the fermenting dough system, higher amounts of titratable acids were formed, and higher pH observed than when higher amounts of malt were added. The lowest pH and highest TA were recorded after 72 hours fermentation of germinated sorghum was due to the high amount of malt in the sample and the enzyme (α -amylase) were available for starch hydrolysis into fermentable sugars. A decrease in pH with a corresponding increase in titratable acidity has been reported in fermentation of various food grains (Abdel haleem *et al.*, 2008; Shimelis and Rakshit, 2008). According to these authors, the production of lactic acid bacteria during fermentation has attributed to the decrease in pH.

4.2. Change in titratable acidity during fermentation

The changes in titratable acidity (TA) during fermentation of sorghum/chickpea blend were presented in Figure 4.2. At the beginning of the fermentation step (0 hours) TA ranged from 0.045% to 0.073% for fermented/ungerminated sorghum (FUS) and fermented/germinated sorghum (FGS), respectively. After 72 hours of fermentation TA increased ranging from 0.108% to 0.153% for fermented/unferminated sorghum/chickpea (FUSC) and fermented/ungerminated sorghum (FGS), respectively. During the fermentation step stating from 0 hours to 72hours TA of all the samples were significantly ($P < 0.05$) increased (Figure 4.2). PH decrease and TA increase are an important parameter for assessing how fast the process will reach conditions (pH lower than 4.5) which can hinder or inhibit the growth of food-borne pathogens. The produced acid during fermentation helps to preserve the products. Fermentation has also been

strongly suggested to have inhibitory effects on the groups of micro-organisms that can cause spoilage or food poisoning (Odumodu and Inyang, 2006).

Figure 4.2 Changes in TA of the sorghum/chickpea mixture during fermentation periods



Reported values are the mean \pm (SE) (n=3). Sample codes are as stated in Figure 4.1.

The pH drop and rise of TA in the present study is attributed to the microbial activity on sorghum (both germinated and non-germinated) and chickpea flour converting some of the carbohydrates in to organic acids such as lactic acid, citric acid, acetic acids and other volatile short chain fatty acids.

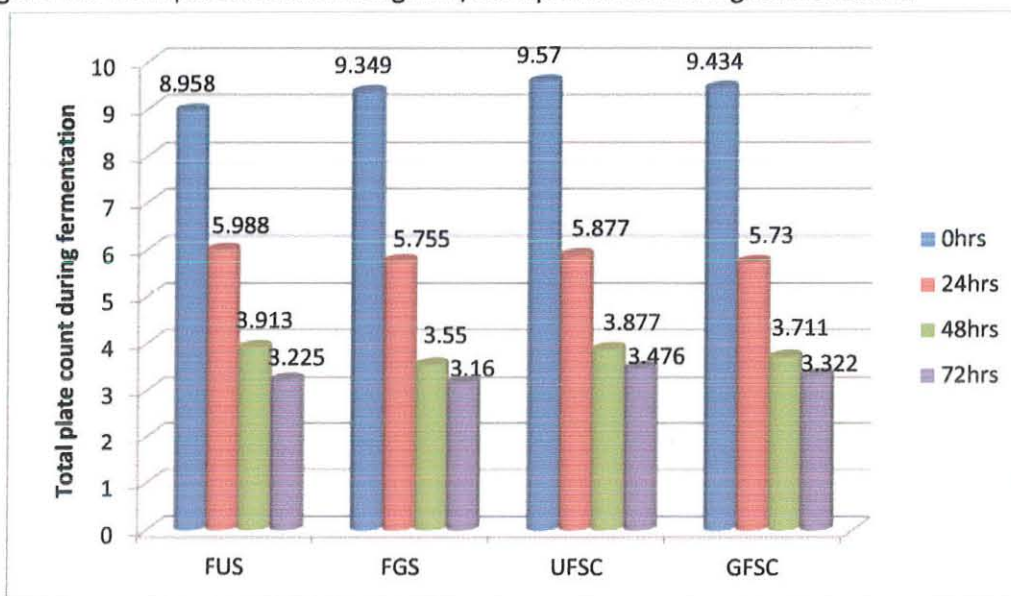
4.3. Microbiological analysis

4.3.1. Total plate counts

To assess the effect of germination and fermentation on microflora (total plate count and lactic acid bacteria (LAB) microbiological analyses were performed during different fermentation steps and the results are presented in (Figure 4.3). The total plate count was

significantly highest ($P < 0.05$) at the beginning of fermentation time; 8.958 logcfu/ml, 9.349 logcfu/ml, 9.570 logcfu/ml and 9.43logcfu/ml for fermented/ungerminated sorghum (FUS), fermented/germinated sorghum (FGS), ungerminated/fermented sorghum/chickpea (UFSC) and germinated/fermented sorghum/chickpea (GFSC), respectively. Within 24hrs hours of fermentation the total plate count drop significantly reaching a maximum of 3.476logcfu/ml after 72 hours of fermentation. The drop of total plate counts were 8.958-3.225 for FUS, 9.349-3.160 for FGS, 9.570-3.476 for UFSC and 9.434-3.322log cfu/ml after 72hrs of fermentation.

Figure 4.3 Total plate count of sorghum/chickpea blend during fermentation



Reported values are the mean \pm Standard Error SE ($n=2$). Sample codes are as stated in Figure 4.1.

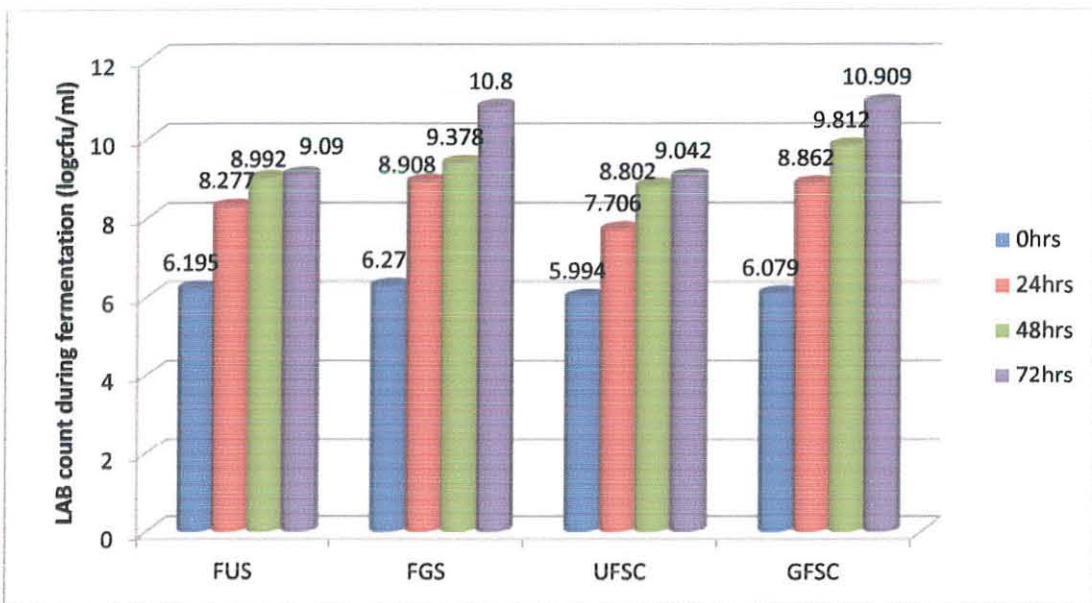
After 48 hours of fermentation the total plate count for all the study samples reached to below an acceptable limit to the preparation of complementary food (≤ 4 logcfu/ml) (Jacqueline et al., 2009) with the result 3.913, 3.55, 3.572 and 3.711 for FUS, FGS, UFSC and GFSC, respectively. Similar observation was made by Nout (1991) on fermented sorghum-based infant food, where the microbes fell from 2.5 to 1.7 log cfu/g after 60 h of natural fermentation. The high initial bacterial count was in the same range as those measured by Sanni et al. (1994) on traditional ogi sorghum. The increased

microbial flora may have developed during the malting process and contamination of the samples during milling, thus resulting in the higher initial count at the beginning of fermentation.

4.3.2. Lactic acid bacterial counts

Lactic acid bacteria count on MRS agar ranged from 4.724logcfu/ml (minimum value) scored for fermentation of non-germinated sorghum flour at 0hrs to 10.909logcfu/ml (highest value) scored at 72hrs of fermentation of germinated sorghum blended with chickpea (Figure 4.4).

Figure 4.4 LAB count of sorghum/chickpea blend during fermentation



Reported values are the mean \pm (SE) (n=2). Sample codes are as stated in Figure 4.1.

The highest LAB count of 10.909logcfu/ml was recorded for sample GFSC at the end of 72hrs fermentation while sample UFSC had the lowest LAB count of 9.042 \pm 0.731logcfu/ml at the same time. The percent of increase of LAB count after 72 hours of fermentation was 31.84%, 33.7%, 41.9% and 44.27% for FUS, FGS, UFSC and GFSC, respectively. Lactic acid bacteria (LAB) population increased during all fermentation steps of sorghum and chickpea blend. Throughout the fermentation process, the predominant microorganisms were lactic acid bacteria with increasing in numbers from

the onset of fermentation to the end of fermentation when compared with total bacterial colonies. This might be due to the ability of the LAB isolates to predominate and suppress the growth of other undesirable microorganisms. Odunfa and Adeyele (1985) also reported domination of lactic acid bacteria in some traditional fermented foods in Nigeria. The predominance of LAB in this study may be due to secretion of lactic acids which inhibits greater growth of other pathogenic bacteria and yeasts in the fermenting medium. Fermentation has also been strongly suggested to have inhibitory effects on the groups of micro-organisms that can cause spoilage or food poisoning. For example, the mean number of diarrhea episodes in preschool children over 9 month period was 2.1 per child using fermented gruels compared with 3.5 per child using non-fermented gruels (Odunfa and Inyang, 2006).

4.4. Proximate composition of germinated and fermented sorghum/chickpea flour

The mean values for proximate compositions such as moisture, crude protein, crude fat, total ash, crude fiber, total carbohydrate and total energy of raw, germinated and fermented states of sorghum/chickpea samples were investigated and the effects of results are presented in Table 4.1.

4.4.1. Moisture content

The proximate composition of sorghum/chickpea flour samples processed by germination and fermentation are presented in Table 1. The mean moisture content of raw, germinated and fermented flour ranged from 5.80-7.90g/100g (Table 4.5). Germinated sorghum (GS) had the highest mean moisture value (7.90g/100g) and fermented/ungerminated sorghum (FUS) had the least value (5.40g/100g). Statistical analysis showed that germination and fermentation had significant effect ($p < 0.05$) on the moisture content of sorghum and sorghum/chickpea blend. As shown in Table 1, moisture content was significantly ($p < 0.05$) increased after germination in both fermented and unfermented samples. During fermentation, also the moisture content of the samples showed significant ($p < 0.05$) increase.

The increase in water uptake during germination is due to the increasing number of cells within the seed becoming hydrated (Oyenuga, 1968).. Low moisture content of food samples is a desirable phenomenon, since the microbial activity is reduced (Oyenuga, 1968). Low moisture content in food samples increase the storage periods of the food products (Alozie et al., 2009); while high moisture content in foods encourage microbial growth; then, food spoilage (Temple et al., 1996).

4.4.2. Crude protein content

The mean protein content of all the study samples of sorghum and sorghum/chickpea blend ranged from 11.42mg/100g for US to 15.787g/100g for GFSC. The mean protein contents of raw sorghum (US) 11.42mg/100g was significantly ($P<0.05$) decreased with value of 12.524mg/100g for germinated sorghum (GS). Also the protein content of raw sorghum/chickpea (USC) blend 13.552mg/100g was significantly lower than 14.912mg/100g germinated sorghum/chickpea blend (GSC). But, germinated sorghum /chickpea (GSC), ugerminated/fermented sorghum/chickpea (UFSC) and germinated /fermented sorghum/chickpea (GFSC) blends had comparable protein contents with the results 14.912, 14.725 and 15.785mg/100g, respectively (Table 4.1).

According to present study germination and fermentation showed significant ($p<0.05$) increase in the protein content of sorghum and chickpea blend, and also fermentation of malted sorghum flour for 22 hrs caused additional increase in crude protein content. Chavan and Kadam (1989) and Ahmed et al. (1991) reported that the observed increase in protein content after processing of samples (germination and fermentation) was probably due to the shift in dry matter content through depletion of carbohydrates; during both germination and fermentation by action of fermenting microorganisms. The increase in protein could be attributed to a net synthesis of enzymic protein by germinating seeds (WHO, 1998); while Tsaio *et al.* (1975) stated that the increase in protein on germination of corn seed was due to mobilization of storage nitrogen producing the nutritionally high quality proteins which the young plant needs for its development. According to Monte et al. (2004) the recommended protein content (grams of protein per 100 kcal of food) for complementary foods is of 0.7 g/100 kcal, from 5 to 24 months. In most countries, the

protein requirements of infants are met when the energy intake is appropriate, except if there is a predominant intake of low protein foods (e.g.: sweet potato and cassava). In the present study all the study samples fulfilled the recommended nutritional intake (RNI) of protein content of complementary foods per day. But, cereals (sorghum) are lack of essential amino acid (lysine, tryptophan), so blending chickpea which is rich source of lysine increased the protein quality of the formulated complementary food.

4.4.3. Crude fat contents

The mean fat content of all the study samples ranged from 3.25 g/100g for germinated/fermented sorghum (FGS) and 5.00 mg/100g recorded for both germinated/fermented sorghum/chickpea (GFSC) blend and ungerminated/fermented sorghum/chickpea (FUSC) blend (Table 4.1). Germination decreased fat content significantly ($P<0.05$) in sorghum samples from the 3.5 to 2.75g/100g and sorghum/chickpea mixture from 4.5 to 4.125g/100g, however, after 48hrs of fermentation the fat content was increased significantly ($P<0.05$) or comparable for both germinated and ungerminated sorghum and sorghum/chickpea blend. Fermentation of germinated and ungerminated sorghum/chickpea blends was comparable on mean crude fat content after 48hrs of fermentation.

The observed decrease fat content during germination might be due to the increased activities of the lipolytic enzymes during germination, which hydrolyse fats to fatty acids and glycerol (Onweluzo, 2009). On the other hand, increase of fat during fermentation may be because of additional release of some lipid ether extractable materials released by fermenting micro-organisms which might contribute to the observed increase in crude ether extract of the samples (Onweluzo and Nwabugwu, 2009). Because of the fat content of germinated/fermented sorghum/chickpea blend is low to fulfill the recommended need of fat from complementary food (15-20%), small amount of oil should incorporated to enhance the fat content of this product.

4.4.4. Crude fiber content

The mean fiber content of ungerminated sorghum (US), germinated sorghum (GS), ungerminated sorghum/chickpea (USC) and germinated sorghum/chickpea (GSC) of non-fermented sample were 7.292, 2.626, 3.046 and 2.442g /100g, respectively. The mean fiber content for 48 hours fermented samples were 2.455, 2.551, 2.741 and 2.471 for non-germinated sorghum (FUS), germinated sorghum (FGS), ungerminated sorghum/chickpea (UFSC) and germinated sorghum /chickpea (GFSC), respectively. The mean crude fiber content of ungerminated sorghum was significantly ($P < 0.05$) higher than germinated sorghum. Also the mean crude fiber content of USC was significantly ($P < 0.05$) higher than the GSC blend. On the other hand, the mean crude fiber content of FUS was higher but not significantly ($P > 0.05$) different from FGS, this was also the same for fermented sample of both UFSC and GFSC blend.

According to this study, germination had significantly ($P < 0.05$) decreased the crude fiber content of the samples. Dicko *et al.* (2006) suggested that germination may increase activity of β -amylase enzyme which hydrolyzes cell wall of carbohydrates during germination. The increase in the activity of β -glucanase enzyme can reduce the fiber content of sorghum. It is reported that during the first stage of barley seed germination process, β -glucanase enzymes degrades the endosperm cell walls and α -amylase degrades starch. Present results in the crude fiber are in agreement with works of Narsih *et al.* (2012) on the study of germination and soaking time to improve nutritional quality of sorghum seed.

4.4.5. Total ash content

Ash content in a food item shows inorganic residues after the organic substance has been burnt away and it gives clue of the total mineral content of a food. The studied sorghum and sorghum/chickpea blend did not significantly vary ($P > 0.05$) in their total ash content during both germination and fermentation (Table 4.1). The mean total ash content ranged from 1.6 to 1.8% for unfermented samples of both ungerminated and germinated samples, and 1.6 to 2.00mg/100g for ungerminated/ fermented and germinated/fermented samples. The present finding is in agreement with the work of (Ramaite, 2004) on the

study of effect of processing (sprouting and fermentation) of five local varieties of sorghum on some biochemical parameters. While, Mubarak (2005) reported that germination and cooking processes caused significant decreases in ash content. Alemu (2009) observed that sorghum ash was significantly decreased after fermentation.

4.4.6. Utilizable carbohydrate content

The mean utilizable carbohydrate content of germinated sorghum (82.105) had significantly higher value than germinated/fermented sorghum/chickpea blend (GFSC) (76.746mg/100g) significantly lower ($P < 0.05$) value. A significant decreased ($p < 0.05$) in carbohydrate levels of the samples was observed with germination and fermentation. This finding was in agreement with the results of Egounlety (2002) which ranged from 71.11-83.39g/100g for nutritive value of high-protein-energy legume fortified flours. The decrease might be due to increase in alpha-amylase activity. The alpha-amylase breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination.

Table 4.1 Effect of germination and fermentation on proximate Analysis of raw, germinated and fermented sorghum/chickpea blend (g/100g)

Sample code	Moisture content	Crude Protein	Crude Fat	Crude fiber	Total ash	CHO%	Energy (Kcal/100g)
US	5.800±0.707 ^t	11.462±0.284 ^t	3.50±0.141 ^{cd}	7.292±0.507 ^a	1.600±0.000 ^b	77.746±0.016 ^d	388.332±1.190 ^{cd}
GS	7.900±0.282 ^a	12.524±0.371 ^e	3.75±0.284 ^c	2.626±0.046 ^{bc}	1.690±0.282 ^{ab}	82.100±0.595 ^a	403.246±0.890 ^e
USC	6.800±0.000 ^e	13.552±0.000 ^c	4.50±0.353 ^{ab}	3.046±0.110 ^b	1.660±0.141 ^{cd}	78.902±0.130 ^{cd}	410.316±0.523 ^{ab}
GSC	7.200±0.141 ^b	14.912±0.124 ^b	4.125±0.141 ^{abc}	2.443±0.000 ^c	1.800±0.282 ^{ab}	78.520±0.196 ^{cd}	410.853±0.473 ^{abc}
FUS	5.400±0.000 ^g	12.745±0.120 ^e	3.75±0.375 ^{cd}	2.551±0.239 ^{bc}	2.000±0.000 ^a	80.964±0.261 ^b	408.586±0.562 ^{bc}
FGS	7.100±0.141 ^c	13.135±0.063 ^d	3.25±0.353 ^{cd}	2.455±0.092 ^c	1.600±0.000 ^c	80.065±0.234 ^b	402.050±2.723 ^d
UFSC	6.856±0.141 ^d	14.725±0.247 ^b	5.00±0.000 ^a	2.741±0.003 ^{bc}	1.650±0.282 ^c	77.534±0.171 ^d	414.036±1.182 ^{ab}
GFSC	6.900±0.141 ^d	15.787±0.124 ^a	5.00±0.000 ^a	2.471±0.007 ^c	1.550±0.282 ^c	76.742±0.035 ^d	415.116±1.131 ^a

Reported values are the mean ±SE (n=2). Means with different superscripts in the same column are significantly different (P<0.05).

US: - ungerminated sorghum flour
 GS: - germinated sorghum flour
 USC:-ungerminated sorghum/chickpea flour
 GSC: - germinated sorghum/chickpea flour
 FUS: - fermented/ungerminated sorghum flour
 FGS: - fermented/germinated sorghum flour
 UFSC: - ungerminated/fermented sorghum/chickpea flour
 GFSC:-germinated/fermented sorghum/chickpea fl

4.4.7. Gross energy

The mean gross energy content of germinated/fermented sorghum/chickpea blend (GFSC) (415.116kcal/100g) was significantly higher when compared to ungerminated sorghum (388.332kcal/100g) which had significantly lower ($P<0.05$) value. The energy value of germinated/fermented sorghum/chickpea (GFSC) blended sample (415.116 kcal/100g) was significantly ($P<0.05$) higher than ungerminated sorghum/ chickpea (410.316Kcal/100g) and germinated sorghum/chickpea (408.586Kcal/100g). So combined processing of germination and fermentation significantly ($P<0.05$) increased the energy content of sorghum/chickpea blend. The value is agreed with the finding of (Oyetero A., 2012) in his work of production and quality evaluation of complementary food formulated from fermented sorghum, walnut and ginger work.

Germinated/fermented sorghum/chickpea (GFSC) had gross energy of 415.116 Kcal/100g (Table 4.1) and with appropriate viscosity of 25% dry matter (DM) content in prepared porridge for young children (Table 4.5). Ungerminated/unfermented sorghum/chickpea (USC) had gross energy of 410.316 Kcal/100g (Table 4.5) and with viscosity of 17% DM content (Table 4.8). Taking into account the gross energy and viscosity of porridges for Germinated/fermented sorghum/chickpea (GFSC) and ungerminated/unfermented sorghum/chickpea (USC), the energy density (ED) of these samples using conversion factor will be $25/100*415.116\text{kcal}/100\text{g}=103.779\text{kcal}/100\text{g}$ for GFSC and $17/100*410.316\text{ kcal}/100\text{g}=69.753\text{ kcal}/100\text{g}$ for USC, respectively. It can be concluded from this study that the processes that include combined fermentation and germination in the case of sorghum /chickpea blend were close to or higher than the minimum energy value of 84 kcal/100g recommended energy density of complementary foods at a rate of 2-4 meals/day added to average breast milk intake (Dewey & Brown, 2003).

4.5. Mineral Analysis of germinated and fermented sorghum/chickpea blend

Because of the rapid rate of growth and development in infants and young children, mineral nutrient has become very essential. For instance, calcification of bone is needed in early infancy to support the weight of the body by the time the baby walks. Also the availability and utilization of calcium in the postnatal period is a crucial factor in adequate tooth formation. Iron is needed especially in the first year of life to prevent iron-deficiency anemia. Zinc is critical during the developmental period because of its role in normal brain development and immune system.

4.5.1. Calcium content

Germination and fermentation were observed to improve the calcium contents of the samples (Table 4.2). In combined form of germinated/fermented sorghum (GFS) and fermented sorghum (FUS), the highest significant ($P<0.05$) calcium levels of 76.155 and 75.855 mg/100g were recorded and in ungerminated/unfermented sorghum/chickpea (USC) blend the lowest 51.495mg/100g significantly value was recorded. Germination alone had also significant ($P<0.05$) effect on the improvement of calcium content of sorghum, but the increase was not significant ($P>0.05$) when germinated sorghum was blend with chickpea. This value is in agreement with the value of (Inyang and Zakari, 2008) in the study of the effect of germination and fermentation of Pearl millet.

The mean calcium content of germinated/fermented sorghum/chickpea (GFSC) is 57.767mg/100g (Table 4.2). According to FAO/WHO, (1988) the estimated need of calcium for young children from complementary food is 353mg of calcium /day. Gastric capacity of young children (12-24 months) is 345ml/meal. If a child feed 345ml of formulated complementary porridge prepared from GFSC; according to FAO/WHO this children gate only about 50% of the needed calcium level from formulated complementary foods. So, calcium from other source (animal source) should be incorporated or supplemented with this formulated porridges.

4.5.2. Phosphorus

During germination and fermentation the mean total phosphorus contents of ungerminated sorghum (22.32mg/100g) was significantly ($P<0.05$) lower and germinated/fermented sorghum/ chickpea (GFSC) had significantly ($P<0.05$) higher (38.87mg/100g). The mean phosphorus content of all study samples were significantly ($P<0.05$) increased during germination, fermentation and combined germination/fermentation processes. This result is in agreement with Ijarotimi (2012) in the study of mineral and nutritional quality of raw, germinated and fermented african locust bean (*parkia biglobosa*) flour.

Table.4.2. Mineral composition (mg/100g) of the sorghum/chickpea blend

Sample Code	Iron	Calcium	Zinc	Phosphorus
US	5.527 ±0.226 ^a	65.455±0.446 ^c	2.323±0.045 ^e	22.32±0.245 ^f
GS	4.870±0.043 ^b	67.694±0.411 ^b	2.507±0.10 ^{de}	25.34±0.098 ^d
USC	5.289±0.195 ^a	51.495±0.134 ^f	2.676±0.026 ^{cd}	26.11±0.168 ^d
GSC	4.601±0.103 ^{bc}	53.534±0.333 ^{ef}	2.608±0.111 ^{cd}	28.78±0.208 ^c
FUS	5.469±0.228 ^a	75.855±0.737 ^a	2.822±0.185 ^{bc}	33.84±0.325 ^b
FGS	4.393±0.018 ^c	76.155±0.412 ^a	2.975±0.000 ^{ab}	38.33±0.028 ^a
FUSC	3.727±0.289 ^d	53.869±0.809 ^e	3.059 ±0.067 ^a	34.85±0.141 ^b
GFSC	4.285±0.024 ^c	57.767±0.897 ^d	3.171 ±0.007 ^a	38.87±0.281 ^a

Reported values are the mean ± (SE) (n=2). Means with different superscripts in the same column are significantly different ($P<0.05$). Sample codes are as stated Table 4.1

4.5.3. Iron content

Iron is needed especially in the first year of life to prevent iron-deficiency anemia. The iron level in the present study was significantly ($P<0.05$) decreased in the combined germinated/fermented process with the decreased value ranged from 5.527 to 4.393mg/100g, for sorghum and 5.289 to 4.285mg/100g for sorghum/chickpea blend, respectively. Germination of sorghum (GS) and sorghum/chickpea (GSC) blend had also decreased significantly ($P<0.05$) compared with un-germinated sorghum (US) and sorghum/chickpea blend (USC) with the result ranged from 5.527-4.87 and 5.289-4.601,

respectively. These results are in contrast to the study of sprouted hungry rice (Nnam, 2000). This is may be due to the difference in the sample. On the other hand fermentation had also decreased iron content of sorghum/chickpea blend, but the decrease was not statistically significant ($P>0.05$). The reduction of total minerals in some of the samples after fermentation may be attributed due to the microbes utilized some of the hydrolyzed mineral elements for their metabolic activities and lost through decantation and /or the minerals could have been lost in the fermentation medium and decant of fermentation water during the drying process (Inyang and Zakari, 2008).

Estimated need from complementary food for iron is (10.8 mg/d) for intermediate (10%) bioavailability; 20.8mg Fe/d when bioavailability is low (5%) and 6.8 mg Fe/d when high (15%) bioavailability based on the basal requirement estimate and (FAO/WHO, 1988). Assuming intermediate bioavailability, the iron content of porridge prepared from GFSC (4.285mg/100g) and gastric capacity for 12-23 months children (345ml/meal) daily iron content of GFSC could be = $4.285\text{mg}/100\text{g} * 345\text{ml}/\text{meal} * 2\text{servings} * 27\% \text{ DM} = 8\text{mg}/\text{day}$. This shows that GFSC meets 74.07% of daily RNI (10.8mg/day) iron as complementary food for breastfeed child aged 12-23months. So, it should be recommended to fortify this product for enrichment of iron to satisfy the RNI per day of iron from complementary food.

4.5.4. Zinc content

The mean zinc content of US, GS, USC and GSC were 2.323, 2.507, 2.676 and 2.608mg/100g for unfermented and 2.822, 2.975, 3.059 and 3.171mg/100g for FUS, FGS, UFSC and GFSC fermented samples, respectively. The value for germinated/fermented sorghum/chickpea blend were significantly ($P<0.05$) highest with exception of UFSC which was not significantly ($P>0.05$) different. Zinc content was significantly affected ($P<0.05$) by germination and fermentation. but not significantly affected ($P>0.05$) by combined form of germination/fermentation. The finding is similar to Abdel-Rahaman *et al.* (2008) except in the case of iron, in which they observed an increase in both total and available amounts of calcium, iron, zinc and phosphorous after 14 hr of pearl millet lactic acid fermentation. Zinc is critical during the developmental

period because of its role in normal brain development and immune system. According to the WHO/FAO (1988) recommended nutritional intake (RNI) of zinc from complementary food is 2.66 mg/d assuming moderate bioavailability of zinc. If we take zinc content of GFSC (3.171mg/100g), daily zinc content from porridge prepared from GFSC would be 6mg/day (>100%) of RNI of zinc for young children.

4.6. Antinutritional factors of germinated and fermented sorghum/chickpea blend

4.6.1. Phytate and phytate mineral molar ratio

The mean values for phytate content among sorghum and sorghum/chickpea blend with different processing methods indicated statistically significant variations. The values ranged from 127.434 to 52.019mg/100g in unfermented samples and 48.233 to 20.096mg/100g in fermented and combined form of germinated/fermented samples. Germination of sorghum for 22hrs significantly ($P<0.05$) decreased the value of phytate from 127.434 to 88.540mg/100g but, significant change was not observed in germinated sorghum/chickpea blend compared with un-germinated sorghum/chickpea blend. Fermentation of germinated sorghum had further significantly ($P<0.05$) decreased phytate contents to the level from 88.54 to 40.840mg/100g (i.e. from 49% to 110% decrement) of the phytate in the samples. Combination of germination and fermentation also had significant effect on the mean phytate contents over fermentation, with value ranging from 48.233 to 40.840mg/100g and from 26.206 to 20.097mg/100g of UFSC and GFSC samples, respectively. The same reports on phytic acid reduction due to germination and/or fermentation of cereals have been documented (Nkama and Gbenyi, 2001; Sutardi and Buckle, 1985). Enzymatic hydrolysis of phytic acid by endogenous phytase of sorghum and/or by phytase which was produced by the microorganism, may account for most of the reduction of phytic acids during fermentation. The low pH of fermented product and temperature of fermentation may also provide favorable conditions for phytase activity (Abdel Rahman and Osman, 2011).

The mean value of phytate: iron molar ratio in the present study was 1.951, 1.538, 0.846 and 0.956 for unfermented samples of US, GS, USC and GSC, and 0.746, 0.786, 0.595 and 0.397 for fermented samples of FUS, FGS, UFSC and GFSC, respectively (Table 4.3). In the present study all the study samples had higher value than the reported phytate: iron critical molar ratio (0.15), indicating that absorption of iron was adversely affected by phytate in both germinated (1.538) and ungerminated sorghum (1.951). But in all other cases the results were found to be lower than the reported critical molar ratio of phytate: iron, indicating that absorption of iron was not adversely affected by phytate in fermentation and combined form of germination and fermentation. However, all other calculated molar ratios in this study such as; Phytate: calcium (>0.24), phytate: zinc (>15) and [phytate x calcium]: zinc (>0.5) molar ratios for all raw, germinated and fermented conditions were found to be less (Table 4.3) than their reported critical values: this indicates that absorption of iron and zinc were not inhibited by phytate and as a result these minerals in all samples are bioavailable. Diets with a phytate: zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with phytate: zinc molar ratio between 5 and 15 have medium zinc bioavailability and those with a phytate-zinc molar ratio less than 5 have relatively good zinc bioavailability (Walingo, 2009).

4.6.2. Phytate phosphorus and nonphytate phosphorus analysis

During germination and fermentation nonphytate Phosphorus significantly ($P<0.05$) increased from 3.03 for raw (control) sample to 33.20 mg/100g for GFSC. Phytate phosphorus was significantly ($P<0.05$) higher in nongerminated/fermented samples compared with germinated/fermented samples. In the present study combined processing of germination/fermentation increased the non phytate phosphorus with corresponding decrease in phytate phosphorus. The percentage of phytate P /total P was 42.90, 26.89 and 17.05% for GFS, UFSC and GFSC samples, respectively. Hence, the lower the

Table 4.3 Anti-nutritional factors analysis (mg/100g) and phytate mineral molar ratios

Sample code	Phytate	Tannin	Phy:Fe	Phy:Ca	Phy:Zn	Phytate P	Nonphytate P	[PhyxCa]:Zn
US	127.43±1.104 ^a	38.467±0.504 ^a	1.951±0.001 ^a	0.114±0.041 ^a	5.435±0.095 ^a	19.28±0.09 ^a	3.03±0.02 ^c	0.0040±0.001 ^a
GS	88.54±1.270	32.574±0.735 ^{de}	1.538±0.042 ^b	0.082±0.028 ^a	3.498±0.520 ^b	19.03±0.24 ^a	6.30±0.47 ^c	0.0031±0.091 ^b
USC	52.91±1.170 ^c	38.354±0.735 ^a	0.846±0.090 ^c	0.062±0.041 ^a	1.958±0.622 ^c	14.91±0.00 ^b	11.19±0.01 ^d	0.0025±0.001 ^b
GSC	52.02±0.952 ^c	36.128±0.586 ^b	0.956±0.061 ^d	0.059±0.081 ^a	1.976±0.241 ^c	14.65±0.14 ^b	14.12±0.14 ^d	0.0023±0.009 ^b
FUS	48.23±0.001 ^d	33.380±0.844 ^{cde}	0.746±0.301 ^f	0.038±0.029 ^a	1.693±0.361 ^d	13.59±0.28 ^c	20.24±0.28 ^c	0.0016±0.109 ^c
FGS	40.84±0.810 ^c	31.857±0.827 ^c	0.786±0.141 ^e	0.032±0.028 ^a	1.360±0.684 ^e	11.50±0.19 ^d	26.82±0.20 ^{bc}	0.0015±0.091 ^c
FUSC	26.21±0.297 ^f	34.694±0.101 ^{bc}	0.595±0.283 ^g	0.027±0.021 ^a	0.848±0.814 ^f	7.385±0.45 ^d	27.46±0.49 ^{ba}	0.0012±0.028 ^c
GFSC	20.10±0.195 ^g	34.197±0.121 ^{cd}	0.397±0.014 ^h	0.022±0.001 ^a	0.627±0.458 ^g	5.66±0.40 ^d	33.20±0.38 ^a	0.0010±0.028 ^c

Reported values are the mean ± (SE) (n=2). Means with different superscripts in the same column are significantly different (P<0.05). Sample codes are as stated in Table 4.5.

phytate phosphorus, the more bioavailable was phosphorus in the germinated/fermented samples. Generally, diets with phosphorus as phytate (%) <60 % are regarded as being adequate in bioavailable phosphate (Melaku *et al.*, 2005). Thus, the hydrolytic reduction of phytic acid during fermentation may have contributed the bioavailability of phosphorus. In raw (controlled) sample there were phytate which lay on the total phosphorus (>60%), this showed poor bioavailability of phosphorus of both sorghum and chickpea in their raw state. The reduction in phytate phosphorus during fermentation may be due to the phytate hydrolysis by phytase elaborated by fermenting microflora (Sandberg and Andlid, 2002; Hurrell *et al.*, 2003). Cleavage of phosphorus from phytic acid may explain the improved availability of phosphorus in germinated/fermented sorghum/chickpea blends.

4.6.3. Tannins

The mean values for tannin content of US, GS, USC and GSC were 38.467, 32.574, 38.354 and 36.128mg/100g, and 33.38, 31.857, 34.694 and 34.197mg/100g for FUS, FGS, FUSC and GFSC, respectively. These values show that germination and fermentation significantly ($P<0.05$) decreased the tannin contents of sorghum and sorghum/chickpea blends. Germinated sorghum with the mean content of tannin 32.574mg/100g were significantly decreased to value of 38.467mg/100g of ungerminated one. Also fermented sorghum (FUS) and combined form of germinated/fermented sorghum (FGS) exhibited significantly ($P<0.05$) lower tannin value of raw and only germinated sorghum (Table 4.3) In a similar manner blending of sorghum and chickpea during germination and fermentation showed significant ($P<0.05$) decrease of the mean tannin content. These results agree with the study of Abdel Haleem *et al* (2008), who reported that natural fermentation of high tannin cultivar (Karamaka) and low tannin cultivar (Mugud) decreased their tannin content by 68.0% and 74.5%, respectively. The reduction in tannins can be attributed to the microbial degradation of polyphenolic compounds during fermentation or due to less extractable tannin-protein complex (Emmambux and Taylor, 2003). The observed reduction in tannin content after germination was a result of formation of hydrophobic association of tannins with seed proteins and enzymes. In addition, loss of tannins during germination also may be due to

the leaching of tannins into the water (Shimelis and Rakshit, 2007) and binding of polyphenols with other organic substances such as carbohydrate or protein (Saharan et al, 2002). In addition during soaking period of germination, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols (Saxena et al., 2003; Khandelwal et al., 2010).

4.7. Viscosity of porridges

At a low flour content (5–15g dry matter/100 ml), the gruel has a free-flowing consistency and is easy to swallow but its energy density (20–40 kcal/100 g) is lower than the minimum value of 84 kcal/100 g of gruel recommended for children aged 9–23 months fed at a rate of 2 meals/day in addition to average breast milk intake (Dewey & Brown, 2003), so that to obtain spoon able (1000-3000cP viscos) porridge for young children, 17% and 25% dry matter (DM) was selected for preparation of porridge. The result for 17% DM concentration showed that, 3438, 808, 3120 and 1605cP for US, GS, USC and GSC of unfermented samples, and 3335, 792, 1958 and 973cP for FUS, FGS, FUSC and GFSC of fermented samples, respectively (Table.4). The viscosity for 25% DM concentration ranged from 6476cP maximum to 1025cP minimum for FUS and FGS, respectively. These result showed that germination had high viscosity reducing effect compared with both fermented and raw samples. The combined form of germination and fermentation showed additional viscosity reducing effect compared with germination. This study results agreed with Firibu (2012) a drastic reduction in peak viscosity was observed as malt concentrations increased. This was probably due to increased starch hydrolysis by the increased enzyme (α -amylase) concentration (Firibu, 2012).

The recommended viscosity of complementary food is in the range of 1-3 p.a.s⁻¹ (1000-3000cP). The viscosity reading range are called drinkable if >1000cP, spoonable if 1000-3000cP and thick if >3000cP, respectively (Mouquet and Trèche, 2001). In the present study porridges had an appropriate consistency with the DM content of 25% for the samples, GS (1436cP), GSC (2445Cp), FGS (1225cP) and GFSC (1986cP) to the young children of 12-23 months age group.

Table 4.4 Viscometer reading of raw, germinated and fermented sorghum/chickpea prepared porridges

Sample code	Viscometer reading at 17% DM (cP) at 45 ° c	Viscometer reading at 25% DM (cP) at 45 ° c
US	3438	6489
GS	808	1436
USC	3120	5122
GSC	1605	2445
FUS	3335	6476
FGS	792	1025
UFSC	1958	4608
GFSC	973	1986

Sample codes are as stated in Table 4.1

4.8. Bulk density of raw, germinated and fermented sorghum/chickpea flour

The bulk density was not significantly different in ungerminated sorghum (0.904g/ml), germinated sorghum (0.912g/ml), ungerminated sorghum/chickpea blend (0.910g/ml) and germinated sorghum/chickpea (0.888g/ml) flour (Table 4.5). These results show that germination had no effect on bulk density of the study samples. Fermentation was significantly ($P < 0.05$) decreased the bulk density with the result ranging from 0.904 g/ml for (US) to 0.725g/ml for (FUS) and 0.910g/ml (USC) to 0.678g/ml (UFSC). Combined effect of germination and fermentation had additional bulk density reducing effect from 0.910g/ml (USC) to 0.639g/ml (GFSC).

The bulk density is a reflection of the load the flour samples can carry, if allowed to rest directly on one another. The density of processed products dictate the characteristics of its container or package product density influences the amount and strength of packaging material, texture or mouth feel (Wilhelm, 2004) . Values obtained from this study were comparable with the values reported by Okaka and Potter (1979). The decrease in bulk density of fermented would be an advantage in the preparation of infant foods. Fermentation has been reported as a useful and traditional method for the preparation of low bulk weaning foods (Desikachar, 1980).

Table 4.5 Bulk Density of raw, germinated and fermented sorghum/chickpea flour

Sample Code	Bulk Density (g/ml)
US	0.904±0.003 ^a
GS	0.912±0.000 ^a
USC	0.910±0.002 ^a
GSC	0.888±0.002 ^a
FUS	0.728±0.001 ^b
FGS	0.658±0.014 ^{cd}
UFSC	0.678±0.009 ^c
GFSC	0.639±0.001 ^d

Reported values are the mean ± (SE) (n=2). Means with different superscripts in the same column are significantly different (P<0.05). Sample codes are as stated in Table 4.1

4.9. Sensory analysis of the sorghum/chickpea porridges

The sensory attributes of porridges made from sorghum/chickpea blends using different processing methods including germination, fermentation and combined forms of germination/fermentation were investigated using 9-point hedonic scale at Addis Ababa University; Center for Food Science and Nutrition by semi-trained panelists of first year M.Sc. program students of Food Science and Nutrition stream and the mean scores of evaluated sensory attributes were presented in Table 4.6.

4.9.1. Taste

Taste is an important parameter when evaluating sensory attribute of food. The product might be appealing and having high energy density but without good taste, such a product is likely to be unacceptable. The observed mean score of taste in experimental porridges ranged from 3.25- 7.80 (Table 4.6). Non-germinated/fermented sorghum/chickpea (UFSC) blend had the highest mean score in taste (7.80) and had significant difference (p<0.05) with US (3.25), USC (3.40) and GSC (4.00) non-fermented porridges but not significant (P>0.05) from GFSC (6.80). According to Nout (1994) listed in Ramaite (2004) fermentation plays a significant role in enrichment of the human dietary through development of a wide diversity of flavors, aromas and textures in food. With exception of unfermented porridges, both fermented/non-germinated sorghum/chickpea blend and

germinated sorghum/chickpea blend scored >6.0 indicating that most of the porridges prepared on average likeable by most of the panelists (Table).

4.9.2. Color

The visual appearance of any food is the first organoleptic sense of a consumer experience. If supplemented food product has any change in appearance, consumers will more likely reject the product due to familiarization of the normal product (Olaoye *et al.*, 2006). Regarding color of the porridges, most of the samples were similar in appearance while only one US (3.75) significantly ($P<0.05$) decreased (Table 4.6). Although, the mean color score results of most of the samples showed non-significant difference, only two of them UFSC and GFSC were in acceptable range (above 5.75) by panelists.

Table 4.6 Sensory characteristics of porridges

Code	Taste	Color	Aroma	Texture	Over all acceptability
US	3.25±0.957 ^c	3.75±0.957 ^b	3.24±0.816 ^c	3.00±0.820 ^d	3.00±0.816 ^d
USC	3.40±1.140 ^c	5.40±0.547 ^a	4.20±0.836 ^c	4.40±0.890 ^c	4.50±0.447 ^c
GSC	4.00±0.710 ^{bc}	5.60±2.073 ^a	6.24±1.000 ^b	6.80±0.836 ^b	5.80±0.836 ^b
UFSC	7.800±1.30 ^a	6.00±0.707 ^a	7.00±1.581 ^a	7.60±1.140 ^{ab}	6.90±0.836 ^{ab}
GFSC	6.80±1.303 ^a	6.20±0.836 ^a	6.60±1.140 ^a	8.20±0.836 ^a	7.20±0.836 ^a

Reported values are the mean ± (SE) (n=22). Means with different superscripts in the same column are significantly different ($P<0.05$). Sample codes are as stated in Table 4.1

4.9.3. Aroma

Smell is an integral part of taste and general acceptance of the food before it is put in the mouth. It is therefore an important parameter when testing acceptability of formulated foods. The mean score of aroma of porridges ranged from 3.24-7.00 (Table 4.6). UFSC had significantly ($P<0.05$) higher mean score in aroma (7.58) while US scored the least mean aroma (3.24). Among the non-fermented samples only GSC (6.24) had scored above 6 (like slightly) suggesting that they are acceptable range by consumers.

Fermented porridges of both non-germinated sorghum/chickpea and germinated sorghum/chickpea scored above 6 which were 7.00 and 6.60, respectively. Thus, combined form of germination and fermentation had significant ($P < 0.05$) effect on the aroma of sorghum/chickpea blend.

4.9.4. Texture

The results revealed that no significant differences ($P > 0.05$) were observed by panelist between UFSC and GFSC in terms of texture and both were highly liked by panelists as indicated by higher scores of 7.00. Except GSC among the non-fermented samples, all others were significantly ($P < 0.05$) decreased in their mean texture score of fermented and combined germinated/fermented samples. The mean score of texture for the non-fermented samples, US and USC had the average score below (< 6) indicating that based on this test parameter these samples were not liked by the panelists.

4.9.5. Over all acceptability

Generally, among the processed porridge products of the sorghum/chickpea, ungerminated/fermented sorghum/chickpea blend (UFSC) and combined form of germinated/fermented sorghum/chickpea (GFSC) were highly acceptable by panelists with mean scores (6.9 and 7.2), respectively. Their acceptability levels were significantly higher ($P < 0.05$) than all of the rest samples. The products of US, USC and GSC were significantly disliked by panelist as compared to the rest of samples, and they scored below the average (< 6).

Chapter Five

5. Conclusions and Recommendations

5.1. Conclusions

This study attempted to investigate the effect of germination and fermentation on low tannin sorghum/chickpea blend to prepare hygienic, sensorial acceptable and energy dense complementary food. It has covered investigation regarding pH, Titratable acidity, microflora, nutritional composition, antinutritional factors, viscosity and bulk density analysis of complementary foods produced from sorghum/chickpea blend.

The result showed that fermentation for 48hrs significantly reduced the total bacterial count to $<4\log_{cfu/ml}$ and pH to the level <4.5 which inhibits the growth of food born microorganisms to the level acceptable to preparation of complementary food. Lactic acid bacteria (LAB) production was significantly increased with fermentation time by dominating the production of other microorganisms.

Fermentation increased crude protein, crude fat and total energy, whereas decreased crude fiber and moisture, but comparable result in total ash and crude fat. Enriching sorghum with chickpea increased the crude protein, crude fat and total energy contents of this sorghum/chickpea based complementary foods.

Combined effect of germinated and fermented sorghum/chickpea (GFSC) has significantly higher values of crude protein, crude fat and total energy; but comparable value of total ash. Decreased moisture and crude fiber content gives additional advantages for porridge prepared from GFSC blended sample. Germination and fermentation were also observed to improve calcium, zinc and phosphorus, whereas iron contents were decreased or comparable with row (control) sample.

This work established that germination and fermentation increased the nutritional bioavailability of the product by significantly decreasing antinutritional factors (phytic

acid and tannins). Moreover, the phytate content and phytate mineral molar ratios of samples were also significantly decreased than raw sample. Thus, germination and fermentation of sorghum/chickpea reduced phytate level, enhance mineral bioavailability and improve nutritional composition of the products.

Combined effect of germination and fermentation had significant viscosity reducing effect on sorghum/chickpea mixture. Fermentation of germinated sorghum/chickpea blend was significantly decreased the viscosity of the porridge from 5122cP for ungerminated/unfermented sorghum/chickpea (USC) blended porridge to 1968Cp for germinated/fermented sorghum/chickpea (GFSC) blended porridge (dropped to acceptable limit of appropriate consistency 1000-3000cP for young children). Among the prepared porridges by germination and fermentation, ungerminated/fermented sorghum/chickpea blend (UFSC) and combined processing of germinated/fermented sorghum/chickpea (GFSC) was highly accepted by panelists.

5.2. Recommendations

- At the household level, germination and fermentation should be practiced, which increases sensory acceptability, energy density and nutritional composition by decreasing food born microorganisms and antinutritional factors of sorghum/chickpea blended complementary foods.
- Because of limited access to nutrient-dense and anti-nutritionally low complementary foods in Ethiopia, this traditionally formulated product should be consumed widely to overcome both acute and chronic mal-nutritional problem in Ethiopia. Stakeholders and government should encourage for further investigation of such a product.
- Comprehensive analysis including amino acid, fatty acid profiles, vitamin complexes and mineral fortifications are required.

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Declaration

I, the undersigned, declare that this is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

Name: TSION MARKOS: _____

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

Mr. Kelbessa Urga: _____

Mr. Tilahun Bekele: _____

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