ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

CENTER FOR FOOD SCIENCE AND NUTRITION

DEVELOPMENT OF CEREALS AND SOYBEAN BASED COMPLEMENTARY FOODS

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

BY

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January, 2014

DECLARATION

I MENGISTU ASFAW, hereby declare that:

(a) This thesis has been written by me and that it is a record of my original research work and has not been presented for a degree in any other University.

(b) All sources of materials used for the thesis have been duly acknowledged.

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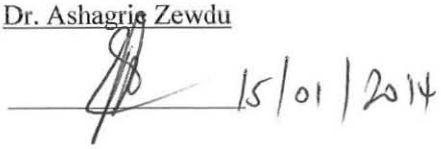
Signature: 

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Approved by the Examining Board

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ACRONYMS

AAS	Atomic Absorption Spectrophotometer
Ab	Absorbance
AHRTAG	Appropriate Health Resources and Technologies Action Group
ANFs	Anti-Nutritional Factors
ANOVA	Analysis Of Variance
AOAC	Association of Official Analytical Chemists
CIMMYT	International Maize and Wheat Improvement Center
CHFIC	Consequences of Hunger and Food Insecurity for Children
Cp	Centipoises
CSA	Central Statistics Agency
CSB	Corn-Soya Blend
DM	Dry Mater
EHNRI	Ethiopian Health & Nutrition Research Institute
ESE	Ethiopian Seeds Enterprise
ESPAGAN	European Society for Pediatric Gastroenterology and Nutrition
ETV	Ethio-yugozlaviya
FAO	Food and Agriculture Organization
HED	High Energy Density
IP6	Inositol Hexa-phosphate
LSD	Least Significance Difference
MSI	Mineral Safety Index
MT	Metric Tons
NCHS	National Center for Health Statistics
NRCFN	National Research Council, Food and Nutrition Board

ABSTRACT

The aim of this study was to produce and evaluate the nutritional quality, antinutritional factors and sensory characteristics of complementary foods from sorghum, maize, soybean, sorghum soybean and maize/soybean blend. Results showed that the moisture content ranged between 4.1g/100g for sorghum/soybean blend to 7.06 g/100g for fermented maize; the total ash content 0.75g/100g for fermented sorghum to 2.6 g/100g for maize/soybean blend; crude protein 7.34 g/100g for maize to 16.73g/100g for fermented sorghum/soybean blend; crude fat content 3.45g/100g for sorghum to 13.3g/100g for sorghum/soybean blend; crude fibre 1.58g/100g for fermented sorghum to 4.4g/100g for fermented sorghum/soybean blend; total carbohydrate 66.39 g/100g for sorghum/soybean blend to 85.56 g/100g for maize and energy value 402.5kcal/100g for sorghum to 442.46kcal/100g for sorghum/soybean blend.Fermentation significantly decreased antinutrient (tannin and phytate) and minerals concentration of the diets. Micronutrient results for Ca, Fe and Zn ranged between 5.64 mg/100g for fermented maize to 231.67 mg/100g for sorghum/soybean blend, 2.5mg/100g for fermented maize to 5.15mg/100g for sorghum/soybean blend and 1.21mg/100g for fermented maize to 3.67mg/100g for sorghum/soybean blend respectively. Result indicated that the antinutrient content (tannin and phytate) ranged between 1.96mg/100g for fermented maize to 31.45mg/100g for maize/soybean blend and 173.4 mg/100g for fermented maize/soybean blend to 362.43mg/100g for sorghum respectively. The calculated molar ratios of diets for phytate: calcium, phytate: Iron, phytate: Zinc and (Ca) (Phytate): Zn ranged between 0.06mM for fermented sorghum/soybean to 2.72mM for fermented maize, 3.83mM for fermented sorghum/soybean blend to 8.68mM for maize, 6.34mM for maize/soybean blend to 20.78mM for fermented maize and 0.02mol/kg for fermented maize to 0.43mol/kg for sorghum/soybean blend respectively. After fermentation the results showed that the pH and titratable acidity ranged between 3.81 for maize to 4.13 for maize/soybean blend and 0.32 for maize/soybean blend to 0.46 for sorghum/soybean blend respectively. The viscosity ranged between 2566cP for fermented maize/soybean blend to 3960.5cP for maize. For the functional property the results showed that the bulk density ranged between 0.58g/ml for fermented sorghum/soybean blend to 0.9g/ml for maize; dispersibility 68.5% for fermented maize/soybean blend to 80.5% for fermented sorghum; water absorption 1.93g/g for fermented sorghum to 2.51g/g for fermented maize/soybean blend and oil absorption 3.54g/g for maize/soybean blend to 4.69g/g for fermented sorghum/soybean blend. Fermentation significantly affects the sensory properties of diets. The results showed that panelists score the color ranged between 6.2 for fermented sorghum/soybean blend to 8.8 for maize; taste 5.4 for fermented maize to 8.2 for maize; aroma 5.2 for fermented sorghum to 7.11 for sorghum/soybean blend; texture 5.8 for maize to 8.4 for fermented sorghum/soybean blend and overall acceptability 6.2 for fermented sorghum to 7.1 for fermented sorghum/soybean blend. Nutritious, acceptable and affordable complementary foods can be formulated using locally available food that can be better than traditional complementary foods. Fermented sorghum/soybean blend diet was concluded to possess the most desirable nutritional profile among formulated food samples followed by sorghum/soybean blend, maize/soybean blend and fermented maize/soybean blend respectively.

Key words: *Fermentation, proximate composition, micronutrient, Antinutrient, Sensory characteristic and complementary foods.*

CHAPTER ONE

Introduction

1.1 Background

Legumes and cereals are the main source of nutrients for complementary foods in developing countries (Charan and Kadam, 1989). Cereals are the source of nutrition for one-third of the world population both in developing and underdeveloped countries of Sub-Saharan Africa and South-East Asia. The major cereals such as sorghum, rice, wheat and maize constitute about 85% of total global cereals production amounting to about 200 million tones of harvest annually at an average of 10% protein content, out of which a sizeable proportion goes into human consumption (Sofi *et al.*, 2009).

Cereals are the most suitable vehicle for delivering amino acids to at risk population due to their frequent consumption, stability and versatility (Bulusu *et al.*, 2007). Legumes such as soybean cultivated in Africa and else are one of the richest and cheapest sources of plant protein that can be good substitute for animal products. Unlike other beans, soy offers a complete protein profile that can be used to improve the diets of millions of people, especially poor and low income society in developing countries because of its nutritional quality, attractiveness and functional properties and it is cultivated for its seeds which are used commercially as human foods, livestock feed and for the extraction of oil (Iwuoha and Eke, 1996).

Cereals and legumes are also rich in micronutrients such as minerals; however, the availability of those micronutrients is usually low due to the presence of antinutritional factors such as phytic acid and tannins. Phytic acid is the most important antinutritional factor and is found in most cereals. Phytic acid has strong ability to complex multi-charged metals ions, such as zinc, magnesium, calcium and iron and makes them unavailable for human body consumption. The simple traditional processing treatments such as fermentation, soaking, germination, roasting and cooking have been used to process the cereals in order to improve the nutrients content by increasing the content of some minerals, vitamins and amino acids (Nadeem *et al.*, 2010).

Malnutrition in children is a major nutritional problem in developing countries which leads to morbidity and mortality, retardation in physical growth and mental development, working capacity and increased risk of adult disease (Michaelsen *et al.*, 2009). There is urgent need for provision of complementary foods that are high in protein, low cost and suitable for children's nutritional needs. However, this is lacking in rural parts of developing countries (Abbey and Nkanga, 1988). Protein-energy malnutrition is a nutritional deficiency disease resulting from an

inadequate intake or utilization of protein and energy. Globally, more than one billion people are undernourished and in Africa there are more than 70 million undernourished children due to poverty and food shortage (Serrem, 2010). This vulnerable population survives predominantly on starchy staples cereals food such as maize, wheat, rice, sorghum, millet with few or no meat and dairy products (Mayer *et al.*, 2008).

The growth of infants and young children in their first two years of life is very rapid and breast feeding only will not be sufficient for the infant's nutritional requirement. After about six months of age, the infant need supplementary feeding particularly food of adequate nutrient density, consistency, and texture, and they need to be fed more often than adults (Achinewhu, 1987). As a result, many brands of proprietary complementary foods has been developed and marketed in most developing countries (Adeyemi *et al.*, 1989). In these countries, children are usually weaned into porridge or gruels prepared from cereal flour such as maize and sorghum which is characterized by bulky, high viscosity and low energy per unit volume of the food, thus necessitating frequent feeding to meet the daily energy requirements of the children. Apart from energy, the food is usually inadequate in other nutrients, leading to widespread protein-energy malnutrition.

Several human nutrition studies conducted in Ghana included that children fed high lysine/tryptophan maize had fewer sick days, a better chance of escaping death due to diarrhea and other infections than those fed normal maize porridge. Stunting is minimized in children weaned on high lysine/tryptophan maize as compared to those weaned on normal maize porridge. Based on these results, it is concluded that high lysine/tryptophan maize holds the promise of improving the nutritional status of vulnerable groups whose main staple is maize and who cannot afford high protein foods to supplement their diets (Vivek, 2008). Breast feeding is nearly universal in Ethiopia ranging from 93% in Addis Ababa to 99% in Harari. Complementary foods are not introduced at appropriate time for many children. At 6-8 months of age, 14% of children continue to be exclusively breast fed, 9% receive plain water in addition to breast milk, 6% consume other water based liquids, 20% consume other milk, and 50% consume complementary foods. The proportion of exclusive breastfed children by age 9-11 months is only 5%. Adoption of recommended breastfeeding and complementary feeding practices and access to appropriate quality and quantity of foods are essential conditions for fulfilling optimal nutrition for infants and young children.

Complementary feeding period is a time when malnutrition starts in many infants contributing significantly to high prevalence of malnutrition in children less than 2 years of age in Ethiopia. Many factors contribute to the vulnerability of children during the complementary feeding period. The complementary foods are often of low nutritional quality and given in insufficient amounts. In Ethiopia poor feeding practices and shortfall in food intake are most important direct factors responsible for malnutrition and illness amongst children (Eschleman, 1984) and a combination of nutritionally inferior diets and inappropriate feeding practices are major contributing factors to development of childhood malnutrition. Complementary feeding improvement should be of highest priority for nutrition of infant and young children because of its crucial role in preventing mortality and enhancing child development (Jacobs and Rubery, 1988).

Generally, complementary foods commonly used are mostly composed of cereals with a limited amount of dried- milk powder. Such mixtures have been shown to be poor in protein content and quality (Achi, 2005). The aim of this study was to develop complementary foods supplemented a cereals and soybean to improve protein and energy content of traditional complementary food especially in rural areas in Ethiopia. The formulation of complementary foods with a variety of cheapest legumes such as soybean has received considerable attention from nutritionists and food scientists in African countries (Uzogara *et al.*, 1990).

Recently, more attention has been directed towards the fulfillment of protein-energy requirements because of widespread occurrence of protein-energy malnutrition and growth retardation in developing countries. The techniques commonly employed in traditional complementary foods development include formulation of high quality protein foods by using cereals and legumes (Omueti *et al.*, 2009a). Legumes are relatively high in lysine, an essential amino acid deficient in most cereals (Nout and Rombouts, 1992). Soybean is frequently incorporated into products used for prevention of malnutrition by improving the nutrient content of foods. Improvement of complementary foods with soybean is a convenient, cheap and highly effective to promote quality of traditional complementary foods. Adding even small quantities of soybean can very much increase protein content and quality of complementary foods (Bekele, 2011).

1.2 Statement of the problem

Complementary food is the process of giving other foods in addition to breast milk when these alone are no longer sufficient to meet the nutritional needs of infants and young children. Complementary foods are important for maintenance and continued growth and development of a child and yet it is often the time when foods are given to provide the volume necessary to keep a child from being hungry without considering the nutritional quality of complementary foods. Traditionally complementary foods are mostly composed of cereals such as sorghum and maize contain a limited amount of nutrients that are low energy density and do not contain enough macro-nutrients and micronutrients to meet the child's daily requirements. The availability of nutrients also low due to the presence of ant-nutritional factors. These foods have been shown to be poor in protein content and quality which cause childhood malnutrition in the form of protein-energy malnutrition. The protein-energy malnutrition is not as deficiency of protein and energy only, but also nutritionally important minerals which are lack in complementary foods and components of key enzymes.

Complementary foods should contain foods of animal source with high biological value to encourage the growth and development of infant and children. However, these foods may not be available to most low-income households in developing countries. Commercially developed or imported complementary foods are not mostly used by low-income households because of high cost and low availability. Therefore, there is a need complementary food with high nutritional quality and acceptability that are comparable with commercially complementary foods which can be prepared easily at home or community from locally available raw materials such as sorghum, maize and soybean. To improve the nutritional quality of traditional complementary foods, sorghum and maize should be supplemented with soybean. Approaches to improving availability include simple technologies such as fermentation that can be applied in the home or community.

1.3 Significance/purpose of the study

- The purpose of this study was to examine nutritional composition, sensory acceptability and anti-nutritional factors of complementary foods prepared from traditional fermented cereals/soybean blend flours.
- To develop complementary foods from cereals and soybean that can be jointly processed to reduce labor, cost energy and loss of nutrients.
- To provide empirical data about the nutritional composition and ant-nutritional factors of both traditionally fermented and unfermented cereals/soybean based complementary foods.

1.4 Objectives of the study

General objective

- The general objective of this study was to develop and evaluate the nutritional quality of complementary foods prepared from sorghum, maize and soybean flours.

Specific objectives

The specific objectives of the research were to:

- To formulate composite blends using sorghum, maize and soybean flour.
- To determine the macro- and micro-nutrient composition of fermented and unfermented sorghum/soybean and maize/soybean blends.
- To analyze antinutrients (phytic acid and tannin) both in fermented and unfermented sorghum/soybean and maize/soybean blends.
- To prepare gruels from the fermented composites and evaluate their sensory properties and viscosity.
- To study the natural fermentation on sorghum/soybean and maize/soybean blends based complementary foods.

1.5 Hypothesis of the study

H_o. Natural fermentation has no effect on the nutritional quality, sensory properties and anti-nutritional factors of sorghum/soybean and maize/soybean blends.

H_1. Natural fermentation has effect on the nutritional quality, sensory properties and anti-nutritional factors of sorghum/soybean and maize/soybean blends.

CHAPTER TWO

Literature review

2.1 Nutrition and health in a child

Good nutrition plays an important role to infancy and early childhood during in life even before birth and period from birth to two year of life. This is for growth, development of brain and maturation of body tissues which occur rapidly during the first year of life. Eating correct nutrients at correct time during growth increases a child's potential. A healthy infant's birth weight doubles by about five month of age and triples by one year and thus infants have a higher basal metabolic rate about twice that of adults, based on body weight (Whitney and Rolfes, 1999).

During early childhood-characterized by rapid growth- it is important that children be provided with an adequate amount of energy. In particular, macronutrients contained in foods that can provide children with energy are fats, carbohydrates and proteins. Proteins are the major functional and structural components for infancy and childhood cells. Excellent sources of high quality proteins are animal liver, meat, fish, cheese, milk, eggs and legumes such as soybeans and green beans. Protein intake is particularly necessary in infancy and childhood when rapid growth requires amino acids to build new tissue especially muscle and organs (Center, 2010).

All amino acids provide nitrogen for synthesis of human proteins, but some indispensable amino acids cannot be synthesized by human body and must therefore be obtained from diet. Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and histidine are essential amino acids in adults. Arginine is an indispensable amino acid for the children. Cysteine, taurine and tyrosine appear to be essential amino acids for the preterm infants, but evidence is inconclusive as to their essentiality for term infants (Cockburn, 1994).

Fats are the second macronutrient essential for guaranteeing a correct and balanced energy level for infancy and children. Fats are a source of energy and essential fatty acids for children. Structural fats are an essential part of cell membrane, neural fabric and overall cellular structure, while stored fats-present especially in adipose tissue, primarily composed of triglycerides-provide a long-term energy reserve for the body. There are two essential fatty acids, linoleic and α-linolenic acids that human body cannot synthesize. These are precursors of phospholipids, prostaglandins and long-chain polyunsaturated fatty acids, including arachadonic and docosahexaenoic acids (Cockburn, 1994).

Arachadonic and docosahexaenoic acids are present in human milk and young infants have limited ability to synthesize both arachadonic and docosahexaenoic acids. Most commercial infant formulas do not contain docosahexaenoic acid, and the membrane phospholipids in the brains of infants whose intake of this fatty acid is deficient have replaced it with other fatty acids. Docosahexaenoic acid is a major constituent of the developing brain, and its replacement with other fatty acids is likely to change the functional characteristics of neural cells (Cockburn, 1994).

Carbohydrates are the third and most important source of energy (in terms of quantity) for the body. Carbohydrates provide significant proportion of the energy to all tissues in the human body, especially the brain and red blood cells which normally utilize glucose as the "fuel" for cell activity. Glucose is an essential fuel for all body tissues and particularly the brain, which is unable to metabolize fat for energy (Center, 2010).

Alongside the main macronutrients, other essential nutrients in a proper diet for preschool- and school-age children are minerals and vitamins. For children, an enough supply of vitamin A is necessary for appropriate development of vision, to guarantee the integrity of epithelial tissue and development of tissue differentiation. The major sources of vitamin A are: liver, dairy products, eggs, fish, and certain types of fruit and vegetables such as carrots and yellow/orange colored fruit (Center, 2010).

Like vitamin A, B vitamin plays a principal role in growth of children, as well as their appropriate sustenance and development. Vitamin C is a key to optimum immune system function and collagen synthesis. In addition, vitamin C contains antioxidant properties and plays a significant role in process of iron absorption. Vitamin D plays an indispensable role in metabolizing calcium (stimulating its absorption in the intestine), muscle functioning, cell proliferation and maturation and correct functioning of the immune system. Other nutrient essential in diet of pre-school- and school-age children are minerals, particularly zinc, iron, calcium, phosphorus, sodium, magnesium and iodine (Center, 2010).

Dietary fibre can be defined as a non-starch or plant polysaccharides that are indigestible by humans. The major components of dietary fibre are cellulose, hemicelluloses A and B, pectin, hydrocolloids, gums, mucilage's and lignin. A common characteristic of all dietary fibre is that they are not fully digested in small intestine and they enter the colon where they are available for fermentation by colonic microflora. The most important positive effect of fibre in children may be that of regulating bowel movement. Some forms of dietary fibre are more excellent than others for increasing stool weight and frequency, softening faeces, increasing faecal bulk and reducing

gastrointestinal transit time. Dietary fibre can prevent and treat constipation and obesity in children (Rolland-Cachera *et al.,* 1999).

High-fibre foods contain a lower energy density, satisfy hunger, "flatten" the post-prandial glycaemic response and slow the rates of food ingestion, gastric emptying and digestion. No adverse effects have been reported from consumption foods containing high fibre in older children and there is no full information obtained from developing countries where higher fibre intakes frequently coexist with low energy intake (Rolland-Cachera *et al.*, 1999).

Foods used for complementary feeding should not generally contain as much fibre as adult diet, because fibre can displace energy-rich foods that children less than 2 years of age need for growth. Infants and young children who receive diets with inappropriately high amounts of low energy-density foods may not have an adequate energy intake, which can result in failure to flourish (thrive). Many foods such as whole-grain cereal products and legumes are a rich source of dietary fibre; however they contain phytates, which impair the availability of zinc and iron from diet (Rolland-Cachera *et al.*, 1999).

Children in households with low incomes are at increased risk for various chronic diseases due to eat few important nutrients to maintain good health. Cultural norms or lack of awareness about healthy eating can also lead to food choices that don't meet nutritional requirement of children. Children have poor nutrition for many different reasons but the negative impacts are magnified by such factors as lack of parental support, social stressors and being uninsured (Bronte-Tinkew *et al.,* 2007).

According to Wardlaw *et al.* (1994) an infant typically increases in length by 50% in the first year. Such rapid growth requires both nourishment and sleep in abundance. Infants also need concentrated source of nutrients and energy to support their tremendous growth and development. However when an infant is inadequately fed, there is a risk of stunted growth and range of biochemical changes that can impair development to the extent of permanently damaging the infant health. Diet influences all facets of a child's growth: physical, mental, cognitive, and psychosocial. Brain development can be restricted by even mild malnutrition but chronic under-nutrition can lead to life-long cognitive limitations and behavioral impairments (Mendoza-Salonga, 2007).

2.2 Protein-energy malnutrition in infants and children

From birth to the age of 4 months, an infant's nutritional needs are perfectly met by breast milk. According to Cameron and Hofvander (1976) breast milk provides infants with all sufficient nutrients for adequate growth and protects them from infections. On other hand, between 4 and 6 months and beyond, breast milk is no longer sufficient to support the growing child and fully cover energy and protein requirements (Brown *et al.,* 1998). When these nutrient requirements are not met, the situation is described as malnutrition. Nutritious complementary foods are therefore needed to be introduced to infants after 6 months of age. However, low nutrient density and high bulk of the complementary foods, early introduction of solid foods, and unhygienic practices predispose infants to malnutrition, growth retardation, infection and high mortality (Eschleman, 1984).

Malnutrition affects physical growth, morbidity and mortality, cognitive development, reproduction, and physical work capacity and contributes greatly to the disability-adjusted life years worldwide. Severe malnutrition in infant and school-aged children exhibits compromised reasoning and perceptual-spatial functioning, poorer school grades, reduced attentiveness and unresponsive play behavior. It shows deficits in intellectual and behavioral functioning, as compared to theirs adequately nourished peers (Mahgoub *et al.,* 2006).

Protein-energy malnutrition is the most serious nutritional deficiency in infants and young children. It is an imbalance between supply of energy and protein, and body demand for them to ensure optimal growth and function. Childhood malnutrition in developing countries is most prevalent between 6 and 18 months of age. Inadequate energy intake of infants in developing countries may be due to limited household food availability. Currently it is the most widespread and serious health problems of children in the world being moderate or severe forms (Organization, 1998). Although infants and children of some developing nations dramatically exemplify this type of malnutrition, it can occur in persons of any age in any country. Inadequate intake of food containing insufficient nutrients leads to under nutrition, resulting in affected of physical growth and health. On the other hand, excess intake of high-energy foods relative to the body needs results in overweight and obesity (Mittra and Kumar, 2004).

Protein-energy malnutrition primarily affects both infants and preschool children and making main cause of growth retardation. About 31% of the children less than 5 years of age in developing countries are moderately to severely underweight, 39% are stunted, and 11% are wasted, based on a deficit of more than two standard deviations below WHO/NCHS reference values (Armar, 1989). It results from inadequate intakes of protein, energy fuels, or both. Deficiencies of protein and energy usually occur together, but when one predominates and deficit is severe, kwashiorkor (primarily protein deficiency) or marasmus (predominantly energy deficiency) ensues.

In Ethiopia approximately 10% of children born will die before their first birthday and 17% will die before their fifth birthday (Girma and Genebo, 2002). According to formulas developed by Pelletier *et al.* (1994), 57 % of under-five mortality in Ethiopia is related to severe and mild to moderate malnutrition (Girma and Genebo, 2002). The consequences of malnutrition observed in children include poor physical development and limited intellectual abilities that diminish their working capacity during adulthood. The economic status of a household is one of the most important factors that determine child nutritional status (Unicef, 1990).

Children in rural Ethiopia are especially very prone to deficiencies of both macro- and micro-nutrients, as they eat from the family dish and often cannot meet their specific nutrient needs. In rural Ethiopia, the diets of the population are predominantly plant-based and low in animal products. Daily consumption of such diets for longer periods may increase the relative risk of nutrient deficiencies and malnutrition (Temple *et al.*, 1996).

Stunting and wasting are the major nutritional problems in the world countries. Its prevalence ranges from 20-40% in Africa and Southeast Asia. In Ethiopia, according to Central Statistics Agency rural nutrition survey in 1992, the highest prevalence of stunting was recorded in South Gondar (74.5%) and the lowest prevalence in South Omo (49.2). Whereas the highest prevalence of wasting was recorded in Tigray (14.2%), and the lowest in Bale (4.4%). Concerning the prevalence of underweight, the highest (59.9%) was recorded in Tigray and the lowest in Bale (29.2%). Generally, the prevalence of moderate and severe forms of stunting and underweight in Ethiopia showed an increasing trend over a decade according to the report on rural nutrition survey in 1992. According to Demographic and Health Survey the national prevalence 51% less than five year children in Ethiopia are stunted and 11% and 47% are wasted and underweight, respectively (Tefera *et al.*, 2005).

2.3 Complementary foods

2.3.1 Definition of complementary foods

Complementary food means any suitable food given to older infants and young children once breastfeeding or infant formula exclusive can no longer meet a child's growing nutritional needs corresponding to a healthy development. Complementary foods are prepared from locally or manufactured that are suitable as complement to breastfeeding or to breastfeeding substitute, when either becomes not sufficient to satisfy nutritional requirements of infant (World Health Organization, 1981).

Complementary food should be: Timely that means starting at 6 months of age; Adequate that means foods should be given in correct amounts, frequency, consistency and using a variety of foods to cover the nutritional intake of child development while maintaining breastfeeding and/or infant formula feeding; Foods should be prepared and given in a safe manner that means to minimize the risk of contamination with pathogens; And they should be given in a way that is appropriate, that means foods are appropriate texture for age of child and applying responsive feeding following the principles of psycho-social care (Dewey, 2001).

Complementary foods are normality a semi-solid food that is mostly prepared in the form of thin porridges or gruels. Development of complementary foods that are characterized by: High nutritional value to supplement breastfeeding, acceptability, low price and use of local food items. During formulation of complementary foods made from locally-available raw materials techniques of food processing, storage and distribution, socio-economic status, cultural and religious factors, sensory properties, food quality and safety issues should be taken in to account (Tizazu *et al.*, 2009).

2.3.2 Importance of complementary foods

As baby grows and becomes more active, infant formula exclusive is no longer sufficient to cover all nutritional requirement and psychological needs of infant. Adapted family foods (transitional foods) are required to fill gap in energy, iron and other essential nutrients between what is provided by exclusive breastfeeding and a total nutritional requirements of infant. This gap increases with age, demanding an increasing contribution of energy and nutrients especially iron from foods other than breast-milk. Complementary foods play an important part in development of neuromuscular coordination, helping to support growth and brain development in infants (Michaelsen, 2000).

The complementary period represents a period of transition from human milk to daily family diet covers a child from 6-24 months of age and is a very vulnerable period. It is a time when malnutrition begins in many infants contributing to high widespread malnutrition in children less than two years of age. In this period, infants are required food with high energy and nutrient densities due to the quantitative and qualitative nutritional requirements of rapidly growth infants are different from those of older children and adults. For example, the requirements of an infant for energy and protein per kilogram body weight are about three times those of an adult (Akre, 1990).

Infants do not have physiological maturity to progress from exclusive human milk directing to family foods. Particularly adapted transitional foods (family foods) are therefore important to bridge this gap, and are required until about 1 year of age when the infants is enough mature to feed normal transitional foods. The introduction of transitional foods also exposes the infants to a variety of textures and consistencies, thus encouraging the development of vital motor ability such as chewing (Michaelsen, 2000).

Full-term babies are born with enough iron to cover their needs in early months and they use their iron store to fill a gap. However, this store is used up to about 6 months. This means complementary foods that provide sufficient iron are important to fill iron gap from 6 months of age to 23 months. If iron gap is not filled, a child will be affected by anemia. The iron gap is high from 6-12 months in a child, so the risk of anemia is highest in this age group. Preterm and low-birth-weight babies are more influenced by anemia because they are born with low iron stores in body; therefore iron gap starts earlier. For most nutrients, the gap becomes larger as child gets older. For calcium, like iron, the gap is larger in the second year (Organization, 2000).

2.3.3 When complementary foods should be introduced

The optimal age at which to introduce transition foods can be determined by comparing advantages and disadvantages of various ages. The sufficient of human milk to provide sufficient energy and nutrients to maintain growth and prevent deficiencies should be assessed, together with risk of morbidity especially of infectious and allergic disease from contaminated food and foreign food protein. Other important considerations include physiological development and maturity, and the various developmental cues that indicate an infant's eating readiness: and maternal factors, such as nutritional status (Michaelsen, 2000).

Complementary foods should be started at 6 months. At this age, frequent human milk should continue despite foods being introduced. After 6 months, breast milk cannot meet all energy and micronutrient requirements of a baby (Agostoni *et al.*, 2008).Thus, complementary feeding is needed to fill the gap between total nutrient needed by baby to grow and the nutrients provided by breast milk. At 6 months a baby digestive system is also mature enough to digest different foods. Finely minced foods will not choke the baby. During the next few months, the variety and amount of foods can be increased, while human milk still continues. When a baby is about 12 months old many of its food requirements can be met by family meals, although continued human milk is recommended to prevent against illness (Figure 2.1). Thus, the period of complementary food stretches from 6 months to about 24 months, after which children stop human milk and their food intake is based largely on family foods (Agostoni *et al.*, 2008).

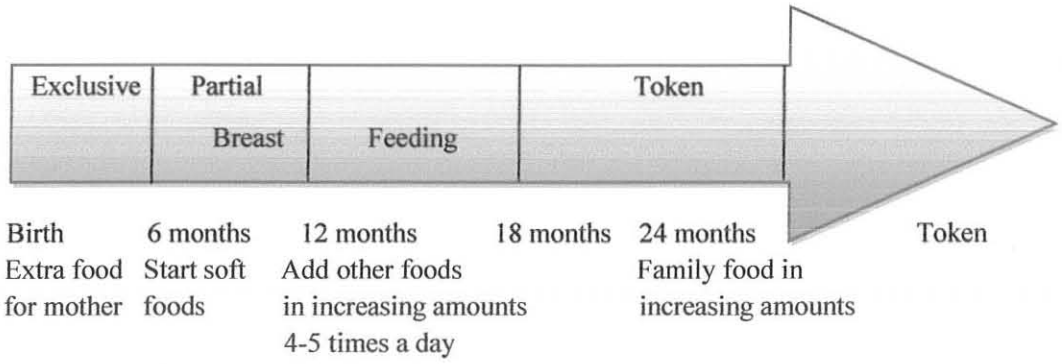


Figure 2.1: Transition of breast milk to family foods (Bekele, 2011)

Supplement of complementary feeding early before 6 months has its dangers because breast-milk can be displaced by complementary foods, leading to reduced production of breast-milk and thereby the risk of insufficient energy and nutrient intake by infants. Infants are exposed to microbial pathogens that are present in foods and fluids since complementary foods may not be as clean as breast milk which is potential source of contamination and thereby increase risk of diarrhoeal diseases and consequently malnutrition (Michaelsen, 2000).

There will also be problems if complementary foods are introduced too late after 6 months because inadequate supply of energy and nutrients from breast-milk alone may lead to growth faltering and malnutrition; micronutrient deficiencies increases such as iron, calcium and zinc, may develop owing to inability of breast-milk to meet requirements, optimal development of motor skills such as chewing and the infants acceptance of new tastes and textures may not be ensured and child stops growing or grows slowly. Therefore it is necessary to introduced complementary foods at the appropriate developmental stage (Michaelsen, 2000).

2.3.4 Nutritional composition of complementary foods

For a newborn infant alone breast-fed, breast-milk provides all required calories for the first 6 months of age. An intake of 95–145kcal/kg (150ml) has been considered adequate. After 6 months of age energy requirement increases for a very placid infant by 32kcal, and a fretful infant by 60kcal (Guthrie, 1989). Complementary foods introduced to infant depend on tradition and availability and thus varies considerably among countries. Gluten is not introduced before age of six months to prevent allergies (Koletzko and Rodriguez-Palmero, 1999) and therefore infant cereals are often based on rice or maize.

As mentioned previously, cereals have low indispensible amino acids such as tryptophan and lysine. In industrialized countries, cow milk is usually added to cereal based complementary foods, either during food preparation as well as during manufacturing of industrially produced infant cereals. In developing countries, where milk is not part of daily diet which is not readily available, cereal-based complementary foods are usually combined with soybean. Soybeans have low essential amino acid such as methionine, however high in lysine. The formulation of cereals and soybean can lead to a well-balanced protein composition (Commission, 2008).

2.3.4.1 Energy density and viscosity

Most traditional complementary foods are usually bulky meaning that food of high viscosity and low energy density and have been found to provide limited energy for growing child (Akinyele and Omotola, 1986). The factors limiting energy intake of an infant weaned on such low energy foods are the volume the child can consume at a time and the frequency of feeding (Walker, 1990).

Viscosity and energy density of complementary foods are important issues to assure appropriate energy intake for infant growth. The required energy density of complementary foods depends on the age of child, the feeding frequency and the amount of human milk consumed. Cereal based complementary foods have high contents of starch, resulting in high viscosity. Work by Onilude *et al.*(1999) indicated that, cereals, in order to be suitable for feeding of young children, are prepared in liquid form by diluting with a large quantity of water, thereby resulting in a large volume with low energy and nutrient density. The consequent low energy density of such foods lead to reduce intake of calories and protein and is an important cause of growth faltering during weaning period from 6-24 months of age.

In developing countries, complementary foods are usually based on foods prepared for adults, i.e. thick cereal and/or legume gruels, diluted with water to reduce the viscosity, resulting

low energy and nutrient density. Low energy density complementary foods caused by high bulk gruels have long been implicated in Protein-energy malnutrition (Okoye, 1992). If the concentration of solids is raised to increase the nutrient and energy density, the gruel will be too thick and viscous for children to eat easily. This high viscosity characteristic is referred to as dietary bulk. Children are not able to ingest them even when not suffering from acute illness. The most efficient way to reduce viscosity, without decreasing energy and nutrient density is by the addition of the starch degrading enzyme amylase (Brown *et al.*, 1998).

The importance of increasing gruel energy density to improve the energy intake of young children in developing countries is now fully recognized (Kenneth, 1997). However, increasing energy density of these generally starch-rich gruels simply by increasing the flour concentration leads to drastic changes in gruel consistency during cooking, i.e. especially substantial thickening. Fermentation is a simple and easily adaptable technology for reduction of bulkiness (high viscosity) and increasing shelf life of cereals and legume based food formulations.

2.3.4.2 Protein

The need for protein during the period of skeletal and muscle growth of early infancy is high. Requirements for protein are about three times higher (per kilogram body weight) during the first months of life than during adulthood. The requirements for essential amino acids are also elevated and would therefore be difficult to satisfy with proteins of low quality (Akre, 1990). However traditional complementary foods in developing countries are low nutritive value (Guiro *et al.*, 1987) and characterized by low protein, low energy density and high bulk. The protein contents of maize and sorghum have poor quality and, are characterized by low in lysine and tryptophan which are two indispensable amino acids to the growth of young child (Oyenuga, 1968).

Indeed, Akinrele and Edwards (1971) concluded that the protein content of complementary foods from cereals was too low even to support the growth of rat. Guiro *et al.* (1987), similarly, noted that the traditional millet gruel used for complementary food for Senegalese children was not energy dense and was insufficient to cover all the nutrient needs of the children. The family diets to which some infants are weaned are also low in nutritional value. Indeed, many investigators (Oyenuga, 1968) have reported that these traditional complementary foods have low protein and the other nutrients are lost due to poor processing.

2.3.4.3 Fat

Dietary fats provide energy for the infant and young child, essential fatty acids and fat soluble vitamins such as A, D, E and K. Fats also heighten the palatability of food thereby promoting greater energy intake. Furthermore, several fatty acids, especially long chain-polyunsaturated fatty acids have specific and essential physiological functions. The different types of fat in the body have diverse function, and the quality as well as the quantity of fat intake is important. Dietary fat intake should range from 30-45% of the total dietary intake for infant and young children less than two years of age (Brown *et al.*, 1998). The largest store of fatty acids that supply long-term energy needs is adipose tissue which is principally composed of triglycerides; similarly, breast-milk fat which is the main energy source for young infants is 98% triglycerides (Guiro *et al.*, 1987)

2.3.4.4 Micronutrients

Nutritionally, the role of micronutrient on health and function of human beings is now well documented. When diet is monotonous, lacks diversity and is largely cereal, legume or tuber-based, the inherent micronutrient content may be low. Even if the micronutrients are present in substantial quantities, as in some cereals or legumes, their bioavailability may be much curtailed due to high phytate content of traditional staple foods, which form insoluble complexes (Brown *et al.*, 1998).The most micronutrients deficient in complementary foods are zinc, iron, calcium and vitamin A (Brown *et al.*, 1998), and are needed in minute quantities for the normal functioning of the body. They are normal chemical components of foods in their active forms or as precursors of the active forms. They form components of enzymes or co-factors needed for metabolic reactions in the body (Devlin, 1992).

Children use their iron stores present at birth in addition to very small amounts of iron provided by human milk for growth and red blood cell synthesis. Therefore, the iron stores are decreasing which lead to vulnerability to iron deficiency after age of six months, if complementary foods do not provide sufficient iron with adequate bioavailability (Dallman, 1992). Combs *et al.* (1996) further stated that absorption and assimilation of iron and zinc are influenced by the antinutrients such as phytates and oxalates which antagonize the uptake of calcium and iron from grains, legumes and vegetables. There have been reports of high prevalence of micronutrient deficiencies among children and women especially in developing countries (Organization, 1995, Organization, 1998).

These deficiencies are increased due to requirements for growth and development, poor bioavailability in plant-based diets, and inadequate attention given to the problem by various community and government. Other identified immediate causes of micronutrient deficiency diseases in children include, inadequate dietary intake, diseases and poor breast-feeding practices, while remote causes include inadequate household food security, basic health services, sanitation and hygiene (Harrison, 1996).

While in industrialized countries commercial complementary foods are often fortified with iron, in developing countries the complementary foods with low iron content and/or poor bioavailability represent a major problem. The situation for zinc and calcium is similar. While animal products have high zinc such as meat and high calcium contents (milk and milk products), plant foods contain less zinc and calcium and poor bioavailability. Vitamin A requirements are also difficult to meet, as retinol is not present in plant foods and ß-Carotine is absent or at low level in most cereals and soybeans (Dallman, 1992).

Vitamins

Vitamins are organic substances that can be found in plants or chemically synthesized in animals, which are required in trace amounts for health, growth and reproduction. When ingested by animals, most of them as the active vitamin or provitamins are modified into co-enzymes that act in concert with enzymes to catalyze biochemical reactions (Zubay, 1993).The scientific evidence supporting the important role of vitamins in promoting health and preventing non-communicable diseases, independent of other nutritional constituents, has been stressed (Organization, 1998).

Vitamin A deficiency is a major public health nutrition problem in the developing world with high risk regions being South and Southeast Asia, Africa etc. The age groups most affected are pre-school children. About 127.2 million pre-school aged children were reported as having Vitamin A deficiency. Vitamin A deficiency is attributed to low dietary intakes of plant sources of vitamin A. The leading disorders include severe infections, increased mortality risk, xerophthalmia and blindness, anemia and poor physical growth.

In recent times, Vitamin A has received global attention due to high prevalence of its deficiency particularly in infants and children. In many parts of the world, efforts to ensure adequate vitamin status for the primary prevention of corresponding deficiencies still continue to receive serious attention. Recommendations offered are focused on the consumption of appropriate (or fortified) foods that provide adequate vitamin A to promote growth, prevent night

blindness and strengthen corneal structure and immune function in children. Folic acid helps to reduce the risk of neural-tube defects as well as anemia and prenatal mortality in women of childbearing age whereas vitamin D promotes bone health in children. Vitamin C, E and certain B vitamins (B6, B12) reduce the risk of cardiovascular and cerebrovascular diseases (Key, 1994).

Minerals

Iron

In foods, iron occurs in two forms such as heme and as non-heme iron. Heme iron present as hemoglobin and myoglobin is absorbed directly as intact iron porphyrin complex. Heme iron is well absorbed (15-35%) and little influenced by physiological or dietary factors (Monsen *et al.,* 1978). The intake of non-heme iron varies widely and, is affected by dietary components and iron status of an individual. Iron deficiency in complementary food is one of the most common global nutrition disorders both in all developed and developing countries. Approximately 50% of children and women of reproductive age and about 25 to 30% of men are iron deficient in developing countries. Iron deficiency even in the absence of anemia can have adverse functional consequences particularly for cognitive development and behavior in children (Yip, 1994).

Iron deficiency anemia (defined as low hemoglobin and two iron status parameters outside the normal range) occurs mostly in infants and children particularly in developing countries, where iron is mostly consumed as non-heme iron. Even with moderate anemia (e.g. 8-9.9g/dl) and severe anemia (<8g/dl), serious interference with the oxygen carrying capacity of hemoglobin and myoglobin can occur. Increased mortality may occur in young children with severe anemia and infection (Yip, 1994).

Zinc

The essential role of zinc in humans' health, growth and development was demonstrated by Prasad *et al.* (1963), based on studies of zinc deficiency in male adolescents manifested as dwarfism and hypogonadism. Zinc is found in various organs, tissues and fluids of body; the total body content is between 2 and 3g (Rimbach *et al.,* 1996). Only a few percent of zinc is in the rapidly exchangeable plasma pool with a small amount of total body zinc approximately 1.5g in children. 60% of the body's zinc is in the muscle, 20% in the bone, 5% in the blood and liver, and 3% in the gastrointestinal tract and brain (Hambidge, 2000).

Zinc is a critical component in over 200 numerous enzyme systems in the body and is involved with Ribose Nucleic Acid (RNA) and Deoxyribose Nucleic Acid (DNA) synthesis, critical to cellular growth and differentiation. Zinc is particularly essential in periods of rapid growth, requiring nucleic acid synthesis and protein metabolism. Zinc deficiency is associated with poor intake of diet, reduced immune response, loss of appetite, impaired taste acuity, skin lesions and a variety of disorders of cell metabolism (Rimbach *et al.,* 1996).

Zinc is absorbed in the small intestine via a carrier mediated transport process or at higher concentrations by passive diffusion (Rimbach *et al.,* 1996). The non-absorbed zinc is excreted in feces. Daily endogenous zinc losses of 1.5-3mg occur mainly via urine, skin and the gastrointestinal tract. Urinary excretion is relatively constant, circa 0.5 mg per day (Sandström, 1997). Body zinc homeostasis is maintained by changes in absorption and endogenous excretion. Changes in excretion were proposed to be the rapidly responding mechanism to small daily variations of zinc intake, while changes in availability are responding more slowly but to larger fluctuations in dietary zinc (Jackson *et al.*, 1984). Infants, fed formulas with low zinc concentrations, showed increased fractional zinc available and decreased excretion of endogenous zinc, demonstrating their ability to maintain body zinc homeostasis (Ziegler *et al.*, 1989).

Calcium

Calcium is important for structural integrity and mineralization of bones and teeth in children and plays an important role in a number of metabolic and regulatory processes. It is a cofactor of multiple enzymes necessary for nerve and muscle function, a component of blood clotting cascade and a regulator of many intracellular processes. A sufficient supply of calcium is vital during skeletal growth to ensure optimum bone mass (Michaelsen, 2000). Milk and milk products are richest dietary sources of calcium. Fish and nuts are also other good sources of dietary calcium. However, the bioavailability of dietary calcium is influenced by binding components such as phytate, oxalate and phosphorus. Breast-milk contains high level of calcium and is sufficient to meet the infant's requirement up to about 6 months of age. Very low intake of calcium in children may results in rickets, growth retardation and biochemical signs of hyperparathyroidism (Thacher *et al.,* 1999).

2.3.4.5. Dietary fibre

Dietary fibre plays an important role in the health of humans and meeting the nutritional needs of animals. Complementary foods should contain maximum level of 5% crude fibre which is suggested by Ijarotimi and Keshinro (2012). This would correspond to >10% dietary fibre, since during sample preparation for crude fibre analysis a large part is removed (Davidsson *et al.*, 1996). Commercial complementary foods are generally low in dietary fibre, since they are often based on low extraction rate cereals flours.

In developing countries, high dietary fibre content is observed in complementary foods which are prepared from both whole grain cereals and legumes. No difference in energy and nutrition intake was found; when health formula fed infants received complementary foods with either low or high dietary fibre contents (Davidsson *et al.*, 1996). Girls and women in each age group have a lower recommended value than do boys or men, except for infants. There are no dietary recommendations for infants, 1 year of age, since it is assumed that most of the nutrients will be provided by milk for the first 6 month of life, and there are no data on dietary fibre intake for infants until after 1year of age. After 1 year of age the children should take 19g/d dietary fibre for both female and males (1-3 years) (Turner and Lupton, 2011).

Table 2.1: Recommended intake levels for dietary fibre (adequate intake, g/d)

Age (year)	Male	Female
1-3	19	19
4-8	25	25
9-13	31	26
14-18	38	26
19-50	38	25
>51	30	21

Source: (Turner and Lupton, 2011).

2.3.5 Sensory characteristics of foods

Sensory evaluation is a scientific method used to evoke measure, analyze and interpret responses to products as perceived through the senses of sight, smell, touch, taste, and hearing. It is an irreplaceable tool in food industry while interacting with key sectors in food production. The sensory behavior of food products is the ultimate criterion for acceptability of any products by consumer. Unless food products meet the desired standards of taste, flavor, texture, etc, the consumer will not accept the product. In other words, quality of food products to a consumer means the sensory behavior of the product (Shimelis, 2007).

When consumers buy any food product, they look for nutritional parameters, like calories, vitamins, minerals, proteins and other ingredients, etc. In sensory evaluation, judges are asked to score the products for color, aroma, taste, texture and overall acceptability using a scorecard of hedonic rating scale. This test is used to evaluate the level of like or dislike for food product by population. Hedonic testing is popular because it may be used untrained people as well as experienced panel members (Shimelis, 2007).

2.3.6 Nutritional problem of complementary foods in Africa and its solution

The most important nutritional problems in complementary foods consumed by infants and children in developing countries are protein-energy malnutrition and micronutrients deficiency (Bukusuba *et al.*, 2008). The high cost and inadequacy in production of protein-rich foods have resulted in increased protein-energy malnutrition among infant and children in developing countries.

Complementary foods are often low nutritional quality and given in insufficient amounts. Poor feeding practices and shortfall in food intake are the most important factors responsible for malnutrition and illness amongst children (Eschleman, 1984) and a combination of nutritionally inferior diets and improper feeding practices are major contributing factors to development of malnutrition in infants and children (Jacobs and Rubery, 1988). Due to the presence of inhibitor complementary foods made from cereals and legume may be low in several nutrients including protein, vitamin A, calcium, zinc and iron. Furthermore, bulkiness of traditional complementary foods, high concentrations of fibre and anti-nutritional factors are major factors in reducing the bio-availability of nutrients.

Complementary foods improvement should be highest priority for nutrition of infant and children because of its crucial role in preventing mortality and enhancing child development (Miller, 1971). Apart from protein and energy, complementary foods of infants in developing countries require more calcium, iron, zinc, vitamin A and D, and other important trace elements. Feeding practice such as time of introduction and type of complementary food quality and quantity of foods given have been identified as one of the most important factors for the child's nutritional status. These can be obtained by combination of commonly used cereals with plant protein sources like legumes can be used to prepare complementary foods for infants and young children which have been proposed by Temple *et al.* (1996).

Poor processing methods and hygiene have also been recognized as other factors responsible for low nutrient density in local complementary foods due to lack of knowledge about simple processing techniques to produce nutritious food (Okoye, 1992). The simple traditional house hold technologies have been used to process the cereal in order to improve the nutritional quality. These include fermentation, soaking, roasting and germination that greatly influence their nutritive value. Of these, fermentation and soaking plays an important role as it influences the bioavailability utilization of nutrients and also improve palatability which can result in enhancing the digestibility and nutritive value (Mariam, 2005).

Fermentation of cereal-legume blend is potentially important processing method that can be expected to improve nutritional value of complementary foods by reducing antinutritional factors and water binding capacity of cereal-based foods (Svanberg, 1987). Fermentation also reduces high bulk density of traditional complementary foods by reducing viscosity of cereals based foods. Incorrect time of introduced complementary food leads to malnutrition and micronutrient deficiencies such as iron, calcium, zinc and vitamin A result child stops to grow or slowly. This is also the other problem observed in infants and children. According to Brown *et al.* (1998) recommended that complementary foods should be start at 6 months. Therefore it is necessary to introduced complementary foods at appropriate developmental stage (Michaelsen, 2000).

2.4 Fermentation

Fermentation is a process by which microorganism's production themselves make use of their external medium as a source of nutrients (Gibson, 1996). It is an old technology and a process dependent on biology activity of microorganisms for production of wide metabolites that can quash survival and growth of undesirable microflora in foods (Klaenhammer and Fitzgerald, 1994). Fermentation is also a method of improving acceptability and sensory quality of many raw materials to such an extent that several foods are preferred in a fermented state compare with

unfermented one. There are furthermore attributes obtained through fermentation such as the reduction of toxic or undesired foods components such as antinutritional factors in legumes (Hammes, 1990).

Fermentation of cereals and legumes with selected microorganisms is a common food preparation method in many African and Asian countries. Many studies have investigated the influence of fermentation on phytic acid content. Loss of phytic acid can be considerable due to the phytase of the microorganisms and or the phytase in the grains or seeds (Mugula, 1992). During fermentation process, a presence of fermentative microbes such as lactic acid bacteria (LAB) causes acidification of fermented food and production of bacteriocins (Gibbs, 1987). As the tolerance of microorganisms to widely differing pH levels varies naturally, the pH selects the species or group of fermentative microorganisms that will be dominant in unaltered food products.

2.4.1 Historical viewpoint of fermentation

Fermentation has been practiced for ancient times in most developing countries, especially in African countries with tremendous variety of fermented foods ranging from those derived from meat and plants to those derived from milk and dairy products (Ray and Daeschel, 1992). Fermentation can be traced back thousands of years and has been used as a method of improving keeping quality of food for more than 6000 years (Holzapfel, 1997). Fermentation, drying and salting are one of the oldest methods of food preservation. Its importance in modern day life is underlined by wide spectrum of fermented foods demanded both in developing and industrialized countries; not only for benefit of preservation and safety, but also for their highly pleasant sensory attributes (Holzapfel, 2002).

Fermentation has enabled our ancestors in temperate and cooler regions to survive winter season and those in tropics to survive drought periods by extending shelf life and improving safety of foods (Campbell-Platt, 1994). Through the ages, fermentation has a major impact on the nutritional habits and traditions, on culture and also on commercial distribution and storage of foods. Traditional fermentation still serves as a substitute where refrigeration or other methods are not available for safe keeping of the food (Holzapfel, 2002).

Fermentation is a traditional methods of storage crops and producing food especially in tropical countries where high temperature and humidity; coupled with unsanitary condition favour food spoilage (van de Sande, 1997). Native fermented food is developed through traditional or village which was preserved over years in order to keep the uniqueness and identity

of these foods (Valyasevi and Rolle, 2002). Over the ages, processes as well as raw materials used have been adjusted to local opportunities and restrictions, the results being a highly diversified traditional food processing system providing income for many people including fermented food producers, retailers and related activity contractors such as millers and transporters (van de Sande, 1997). Food scientists are completely sure that they are able to improve traditional fermentation processes based on their scientific understanding of fermentation processes. They have also proven the ability to influence the duration of fermentation processes, the taste and food value of end products. To date, lactic fermentation of food is carried out through traditional village-art methods (Odunfa and Oyewole, 1997).

2.4.2 Effects of fermentation on foods

Fermentation is responsible for sensory and nutritional properties of diverse foods and can thus result improved flavors, color, texture and aroma. Fermented foods often have definite sensory characteristics as compared to unfermented foods. The aroma and flavor compounds of fermented foods may include acids; carbonyl compounds esters, ethanol and ketones, etc. During fermentation both texture and other sensory characteristics may be altered; for example cereals into bread and soybeans into soy sauce. The fermentative action of microbes may bring about a significant nutritional improvement including increases in protein content especially amino acids (Ramaite, 2004).

Fermentation can improve protein levels of high-starch substrates and also cause loss of starch solids resulting to doubling of protein content on a dry mass basis (Ramaite, 2004). Fermentations of cereals/legumes formulation are especially nutritionally important because the balance of amino acids is often improved. The process of fermentation can also lead to improvements in some vitamins such as in sorghum and maize beer fermentation of Southern Africa, the increase in levels of thiamine in Indonesian Tampe fermentation of rice and vitamins B_{12} in Korean kimchi vegetable fermentation and Indonesian Soybean Tempe fermentation. Most fermented foods supply a valuable source of readily available energy, glucose, maltose, ethanol and organic acids. The process of fermentation also lead to a nutritionally more advantageous balance of fatty acids with increases in levels of polyunsaturated acids (Nout and Rombouts, 1992).

2.4.3 Effect of fermentation on pathogenic microorganisms

Over a study period of nine months, a group of children fed with lactic acid fermented gruel had average of 2.1 diarrhoea episodes compared with 3.5 average recorded for the group fed with unfermented gruel (Lorri and Svanberg, 1994). Although Salmonella, Campylobacter, Shigella, Vibrio, Yersinia and Escherichia are the most common organisms associated with bacterial diarrhoea diseases, other enterotoxigenic genera, including Pseudomonas, Enterobacter, Providencia, Achromobacter and Flavobacterium, have also been reported (Nout *et al.*, 1989). In addition, it was found that there was no significant difference between the behavior of pathogens in fermented porridge or acid-supplemented non fermented porridge, which indicated that the anti-microbial effect is due to presence of acetic and lactic acids at reduced pH, and that other anti-microbial substances do not play a detectable role (Nout *et al.*, 1989).

Similarly, lactic acid bacteria are inhibitory to many other microorganisms when they are cultured together, and this is the basis of extended shelf life and improved microbiological safety of lactic-fermented foods (Adams, 1990). Lactobacillus species can produce a variety of metabolites, including lactic and acetic acids which lower pH, that are inhibitory to competing bacteria, including psychrotrophic pathogen (Breidt and Fleming, 1997).

2.4.4 Food safety aspect of fermented foods

Approximately more than 13 million infants and children under five years of age die annually in tropical regions of world. Diarrhoeal diseases are the commonest illnesses and have greatest negative impact upon growth of infants and young children. The causes of diarrhoea have traditionally been ascribed to water supply and sanitation (Motarjemi *et al.*, 1993). Foods prepared under unhygienic conditions and often heavily contaminated with pathogenic organisms play a major role in child mortality through a combination of poor nutrient absorption, malnutrition and diarrhoea diseases.

All food items contain different types and different amounts of microorganisms. The types of microorganisms that will dominate depend on several factors, and sometimes microorganisms initially present in very low amount in food, for example lactic acid bacteria (LAB), will outnumber, the other organisms inhibiting their growth. In contrast to fermented meat, fish, dairy and cereal products, fermented vegetables have not been recorded as a significant source of microbial food poisoning (Fleming and McFeeters, 1981).

2.4.5 Fermented foods in Africa

Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes; particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids to non-toxic products with flavors, aromas and textures pleasant and attractive to the human consumer. However, if the products of enzyme activities have unpleasant odors or undesirable, unattractive flavors or the products are toxic or disease producing, the foods are described as spoiled (Steinkraus, 1996). Traditionally, the raw materials used for fermentation are diverse and these include fruits, cereals, honey, vegetables, milk, meat and fish. It is possible to obtain a large variety of different food products by selecting different raw materials, starter cultures and fermentation condition (Hansen, 2002).

Fermented foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the foods (Campbell-Platt, 1987). Fermentation is native to Africa countries and has been habited for many centuries (Oyewole, 1997). Therefore, in Africa fermented foods are said to constitute a major portion of peoples diets (Sanni, 1993). A large number of fermented products in Africa are cereals-based and the products include porridges or gruels, bread and alcoholic and non-alcoholic beverages (Oyewole, 1997). The major cereals production in Sub-Africa includes maize, rice, millet and sorghum (Table 2.2). Cereals are more widespread utilized as staple food in Africa countries than in developed world and account for as much as 77% of total caloric consumption. They also contribute substantially to dietary protein intake in a number of these countries.

A majority of traditional cereal based foods consumed in Ethiopia are processed by natural fermentation which is important especially as complementary foods for infants and children as well as for adults as dietary staple foods. These fermented cereal-based food products can be classified on the basis of either the raw cereal ingredients used in their preparation or the texture of the fermented products. In Ethiopia the major fermented cereal-based foods are derived mainly from maize, sorghum and millet (Boling and Eisener, 1982).

Table 2.2 Production of cereals (metric tons) in Sub-Africa

Cereals	1997	% of world production
Maize	24,798	4.2
Millet	10,950	38.9
Rice	11,321	2.0
Sorghum	17,400	28.2
Wheat	3,140	0.5

Source: (Ramaite, 2004)

Fermented foods are essential components of diet in a number of developing countries which are available either as main dishes or as condiments (Steinkraus, 1996). They are prepared from plant materials by using processes in which microorganisms play an active role in nutritional and organoleptic modification of starting materials (Aidoo, 1995). However, due to shortage of scientific and technological understanding fermented foods are generally evaluated on the basis of qualitative attributes like flavor and aroma (Valyasevi and Rolle, 2002).

As compared with foreign imported foods, the traditional indigenous foods sometimes lack appeal because of their presentation, unhygienic production practices and irregular quality. The prestige of traditional foods among these groups is low, and thus in many cases lead to their substitution by the more prestigious foreign imported foods (Nout, 1981). Upgrading traditional processes may provide an alternative far superior to food imports because it would avoid the dependency of the urban populations on expensive foreign foods, maintain employment opportunities in the sector itself reduce logistical problems in food distribution and also provide farmers with an opportunity to sell their product locally (van de Sande, 1997).

Generally, fermented foods are used as complementary products that have benefits for enhancing the nutritive value and food safety. Fermentation can reduce high bulk of unfermented products by reducing viscosity of cereal gruel and hence increases density of nutritional value and energy intake (Graham *et al.*, 1986). This is important since the volume of traditional diets is too large to allow the child to ingest all food necessary to cover its energy needs. Cereal based diets have lower nutritional value than animal based ones; however, meat is often not an option in Africa and use of fermented food seems to be the best option available (Michaelsen and Friis, 1998).

Table 2.3 Summary of most widely used fermented cereal complementary foods in Africa.

Country	Food name	Description
Nigeria	Ogi, Pap, Akamu, Koko	Fermented maize or sorghum
Ghana	Koko, Kenkey	Fermented millet or maize porridge
Sierra Leone	Ogi, Couscous ogi	Fermented maize or sorghum gruel
Benin	Ogi	Fermented maize, sorghum or millet gruel
Burkina-Faso	Ben-saalga	Fermented millet porridge

Source: (Onofiok and Nnanyelugo, 1998).

2.5 Cereals and soybean

2.5.1 Production of cereals in the world, Africa and Ethiopia

Cereals belong to grass family (Gramineae) that have been collected and cultivated for theirs quality and size of grains for many years and are grown globally as main crops (Redhead and Boelen, 1989). Cereals are one of the most important food and cash crops for the majority of Ethiopian farmers and rural households. Maize, sorghum and wheat supply over 50% of average daily caloric intake. The production of cereals accounts for roughly 60% of rural employment and 80% of total cultivated land. Households spend an average of 40% of their total food budget on cereals (Epar, 2010).

Maize is instrumental for the food security of Ethiopian households, and is the lowest cost caloric source among all major cereals, which is significant given that cereals dominate household diets in Ethiopia. Maize is the largest and most productive crop in Ethiopia. In 2007/08, maize production was 4.2 million tons, 40% higher than teff, 56% higher than sorghum, and 75% higher than wheat production. With an average yield of 1.74 tons per hectare (equal to 3.2 million tons grown over 1.8 million hectares) from 1995 to 2008, maize has been the leading cereal crop in Ethiopia since the mid-1990s in terms of both crop yield and production. Wheat and sorghum yields have averaged 1.39 and 1.36 tons per hectare, respectively (Rashid *et al.*, 2010).

2.5.1.1 Overview maize (Zea mays) production in world and Africa

There are many different varieties of maize available, such as Zea mays (flint corn) or Zea mays dentiformis (dent corn), special breeding such as high-oil or high-protein maize (Food and Nations, 1992) and Zea mays saccharata (sweet corn), which contains more sugar than starch in upper endosperm, however all maize is classified as Zea mays. Maize originated from Central

America from where it spread northwards and southwards, and maize also in Europe after discovery of American continent. Maize is grown in almost every agricultural region of the world particularly; North and Central America are the biggest maize cultivated in the world production (Food and Nations, 1992).

Maize is used for human diet, animal feeding and it serves as raw material for production of starch, oil, protein, alcoholic beverages and food sweeteners (Food and Nations, 1992). Many different staple foods can be prepared for human consumption such as gruels, polenta, corn flakes, corn bread or popcorn, depending on area. Fermented maize is mostly consumed in Africa, where it is also used for preparation of brewing beer. Maize flour is widely used for the preparation of tortillas in Central and South America after alkaline treatment (Food and Nations, 1992).

2.5.1.2 Overview sorghum production in world and Africa

Sorghum and millet belongs to small-grain cereals. Sorghum bicolor also called Sorghum vulgare which grow in South Africa, Sorghum guineense in West Africa, Sorghum dura in North Africa. India and China, Sorghum halepense in the Philippines (Redhead and Boelen, 1989). Sorghum saccharatum (sweet sorghum) mainly grows for sugar content of stem, as a substitute to sugar cane, and it is now used to production of molasses 'sorgho syrup" (Redhead and Boelen, 1989). Sorghum originated in North Africa and spread throughout Africa and the Middle East and later on to China and America.

Sorghum is grown in latitudes below 45° in all continents, with the USA. India and China are the major producers (Kent and Evers, 1994). The importance of sorghum in Ethiopia can be adjudged from the fact that there is an almost variety of Ethiopia traditional sorghum food. These include: bread, porridges, gruels (Murty and Kumar, 1995). Between 2006 and 2008, Ethiopia produced an average of 2.5 million MT of sorghum per annum.

In the USA, Australia, Japan and Europe, sorghum is almost entirely used for animal feeding, while in Africa and India it is mostly used for human diet (Kent and Evers, 1994). Sorghum grains are hard and the outer bran layer is bitter and astringent which are removed by milling. Traditional home processing, sorghum is soaked prior to removal of bran. Raw and fermented sorghum are used for preparation of complementary foods such as gruel and porridge. The color of sorghum grains may be white, yellow, brown or red. However, the protein availability and digestibility are reduced in darker varieties of sorghum due to contain more

tannin in husk and therefore the white varieties are most palatable. For beer brewing the dark-colored grains are acceptable (Kent and Evers, 1994).

2.5.1.3 Nutritional composition of cereals

Cereals are staple food in majority of countries which is providing a major source of nutrient such as carbohydrate, dietary protein and fibre for world's population. From minor constituents' B-group vitamin and minerals like potassium, phosphorus and magnesium are the main ones. The chemical composition of cereals grain is characterized by high content of starchy, a substance with a relatively significance protein content and a relatively low lipid content (Goldberg, 2008).

Nutritional value and sensorial characteristics of cereals products are sometimes inferior (not good) or poor in comparison with milk and milk products. The reasons behind this are lower protein content; lack of two essential amino acid such as tryptophan and lysine, low starch availability, presence antinutritional factors like phytic acid and tannin and the coarse nature of the grain (Chavan *et al.*, 1989). Most cereal grains have similar chemical composition and nutritive value. Depending on species, variety and cultivation, carbohydrate content is found between 60-90%, protein content 5-15% and fat content from 1-5% (Redhead and Boelen, 1989).

Carbohydrate

Cereals are important components of daily diet providing carbohydrate-rich foods, as they are composed of approximately 75% carbohydrate. Starches, a major component of cereals, occur in starch granules in endosperm. The ratio of amylose to amylopectin within starch granules varies, depending on cereal and its variety. This is referred to as resistant starch and appears to act in a similar way to dietary fibre. Four categories of resistance have been defined (Baghurst *et al.*, 1996). A small amount of free sugars is also present approximately 1-2% mainly sucrose but low concentration of maltose and very low concentration of fructose and glucose occur.

Protein

Cereals have usually about 6-15% protein (Goldberg, 2008). Cereals supply a good range of amino acids, a building block of protein and some are present in relatively low amounts. The essential amino acid that is in shortest supply in relation to need is termed limiting amino acid. In cereals a limiting amino acid is lysine, except for rye, where tryptophan is a first limiting amino acid (Macrae *et al.*, 1993). More favorable essential amino acid composition can be found in rice, rye, barley and high-lysine cultivars (e.g. maize, sorghum and barley). Sorghum contains around 11-13% protein but sometimes higher values are reported (Dendy, 1995). The protein contain of maize about 9-10% with deficient of two essential amino acids. Normal maize is deficient in

tryptophan and lysine amino acids. Protein related characteristics of maize grains were studied on quality protein maize genotypes which reported 8-11% protein (Vasal, 2002).

Lipid

The lipid components of cereal grains represent a small constituent compared with protein and carbohydrate. Lipids are only a minor component of cereals, with amount varying from a lipid content of 1-3% in barley, rice, rye and wheat, to 5.9% in corn and 5-10% in oats on a dry matter basis (Southgate and Johnson, 1993). The lipid component of sorghum grains represent a small constituent compared with protein and carbohydrate. Crude fat content of sorghum averages about 3% which is higher than that of lipid content of 1-3% in barley, rice, rye and wheat and lower than lipid content of 5.9% in corn and 5-10% in oats on a dry matter basis (Southgate and Johnson, 1993).

Vitamin

Cereals are an important source of most vitamins B, especially thiamin, riboflavin and niacin (Kulp and Ponte Jr, 2000). Cereals are also rich in vitamin E. However, Cereals lack vitamin C, vitamin B_{12}, vitamin A, and apart from yellow corn, no beta-carotene (Cordain, 2004).

Minerals

In cereals, about 95% of minerals are sulphates and phosphates of magnesium, potassium and calcium. Cereals are low in sodium and good source of potassium, in common with most plant foods contain considerable amount of iron, magnesium and zinc as well as lower levels of many trace elements, such as selenium. Maize is a good source of iron, zinc, copper, and manganese (Guria, 2006). Sorghum is considered a good source of potassium, with contain considerable amount of iron, magnesium, zinc, copper and low in sodium. However bioavailability of iron in sorghum is negatively affected by the presence of polyphenols and phytates (Lyons *et al.*, 2003).

Table 2.4: Nutritional value of grain cereals (g/100g)

Cereals grain	Protein	Lipid	Starch	Fibre	Ash
Wheat	12.2	1.9	71.9	1.9	1.7
Rye	11.6	1.7	71.9	1.9	2.0
Triticale	11.9	1.8	71.9	1.9	1.8
Barley	10.9	2.3	73.5	4.3	2.4
Oats	10.2	5.8	55.5	10.9	3.2
Maize	10.2	4.6	79.5	2.3	1.3
Millet	10.3	4.5	58.9	8.7	4.7
Sorghum	11.0	3.5	65.0	4.9	2.6
Rice (brown)	8.1	2.3	75.8	1.8	1.4

Source: (Lasztity and Lasztity, 1990)

2.5.2 Overview soybean (Glycine max) production in world, Africa and Ethiopia

Soybean (Glycine max) originated in orient, probably in China (Snyder and Kwon, 1987). In orient, oil and meals are the main products from soybean. In addition to this soybean is a fascinating (very interested) for achieving food and nutritional security for both poor producer and consumer. Although soybean is served as food in orient over centuries, the amounts used currently are not as great as one might expect, as even China, the home country of soybean (SB), imports to meet its demand. The annual consumption is reported to be 15-20kg per person in all forms (Wang *et al.,* 1979) in China.

Like most food crops grown in Africa, the production of soybean are mainly rain fed. Soybean is generally grown by small-scale farmers on small land areas and in various mixed cropping systems, usually with little or no input. Soybean grows from sea level up to 2000m from equator to latitude 55°N and 55°S. The soybean grow under a wide range of temperature, however the optimum for growth and development is 30°C whilst for proper emergence of seedlings, a seedbed temperature of 25-33^{0}C is optimal (Coulibaly *et al.*, 2009).

Soybean entered to Ethiopia 50 years ago. Till now there have been a number of different soybean varieties studies are conducted. Through studies it has been observed suitable conditions and places for growth of been, suitable plantation periods and technique of production and productive varieties are well determined (Muhsin, 2009). Soybeans are an annual crop which are easy to grow and produces more protein and oil. It is a versatile food plant that used

in its various forms, is capable of supplying most nutrients. Soybean protein quality has been the subject of intense investigation for several decades due to soybean's increasing importance as human food resource (Yimer, 2008).

2.5.2.1 Nutritional composition of soybean

Soybeans are very rich source of protein, lipid and other nutrients. Soy protein products can be good substitutes for animal products because, unlike some other beans, soybean offers a complete protein profile. Besides all essential amino acids are present in soybean except methionine which must be supplied in diet because it cannot synthesized by human body. Although carbohydrates are major constituents quantitatively, they play a minor nutritional role. This is due to soybeans are consumed more for their protein content and value, than for their carbohydrate contribution to human diet (Snyder and Kwon, 1987).

Carbohydrate

The carbohydrates present in soybeans are called complex carbohydrate, starchy vegetables and whole grains. Soybeans are high source of indigestible fibre carbohydrate and an important source of energy for body metabolized into glucose during digestion. Carbohydrates make up approximately 35% of soybean. Approximately 50% of soybean carbohydrate are non-structural in nature and include low molecular weight sugars, oligosaccharides and small amount of starch (Karr-Lilienthal *et al.*, 2005). The other half comprises polysaccharides that include considerable amount of pectin polysaccharides. Small amount of free galactose, glucose, fructose and sucrose make up low molecular weight sugars. Galacto-oligosaccharides (raffinose, starchyose and verbascose) comprise approximately 5% of soybean dry matter, while starch represents less than 1% (Karr-Lilienthal *et al.*, 2005).

Protein

Soybean is considered by many agencies due to a source of complete protein and its protein is valuable because it has an amino acid composition that complements with cereals. Soybeans are relative low sulphur containing amino acids such as cysteine and methionines but contain enough lysine to overcome lysine deficiency of cereals (Potter and Hotchkiss, 1998). The content of protein in soybeans are 38-44% which is larger than protein content of other legumes, 20-30% and much larger than that of cereals, 8-15% (Snyder and Kwon, 1987). This large content of protein in soybeans along with high biological value increases their value as feed stuff and is one reason for soybeans have higher economic advantage compare with other oil seeds.

Fat

Most fat present in soybean is unsaturated. Soybean oil provides calories which are essential fatty acids, vitamin A and E, but provide insignificant amount of vitamin D and K (Bates and Matthews, 1976). Soybean contains high fat content, ranging from 12 to almost 30% (Keshun, 1997). Soy oil can serve as a good source of polyunsaturated (primarily linoleic acid), monounsaturated (oleic acid) and saturated (primarily palmitic acid) which is an essential fatty acid (EFA). Soy oil containing 25% linoleic and 3% linolenic acid (Potter and Hotchkiss, 1998). Polyunsaturated fat content of soybeans is interesting due to it includes linolenic acid (7% of total fat content), an omega -3 fatty acid. Omega -3 fatty acids are essential nutrients for infants and help for health beneficial to reduce risk of cancer and heart disease.

Vitamin

Soybean is rich in vitamins and contain sufficient amount of fat-soluble vitamins (Singh, 2010). The water-soluble vitamins of soybean mainly include thiamine, riboflavin, niacin, pantothenic and folic acid. Vitamin C is negligible in mature beans, but it is present in measurable quantity in immature and germinated beans (Bates and Matthews, 1976). The main oil soluble vitamins include vitamin A (retinol) and E (tocopherol). The tocopherol content of soybean varies with variety. The vitamin D and K content is negligible. Vitamin A exists as pro-vitamin β-carotene. Like vitamin C, its content is negligible in mature bean, but is measurable in immature and germinate seed (Bates and Matthews, 1976).

Minerals

Soybeans are rich in minerals. One cup of soybeans provides 18% of daily value for calcium, 37% of magnesium, 42% of phosphorus, 25% of potassium and 49% of iron. Like other components, minerals in soybean are influenced by variety, growing, location and season (O'Dell, 1979). Generally, mineral bioavailability from consumption of animal food is better than from plants foods (Cook *et al.*, 1981). Calcium, zinc and phytate in soybean foods interact to form a highly insoluble complex, which reduce zinc absorption to a greater extent than phytate alone (Cook *et al.*, 1981).

Table 2.5: Composition of various foods (g/100g)

Food	Protein	Lipid	Carbohydrate	Moisture
Soybean	45	25	15	7
Peanuts	25	50	20	9
Pea	23	2	70	12
Field bean	30	2	60	12
Wheat (grain)	14	2	78	10
Maize	10	5	80	10
Sorghum	11	4	81	11
Cassava	3	0.8	90	63

Source:(Cheftel *et al.*, 1985)

Dietary fibre

Dietary fibre present in soybean plays an important role in many physiological processes and prevention of different diseases origin (Rodríguez *et al.*, 2006). The insoluble carbohydrate in soybeans consists of complex polysaccharides, such as hemicelluloses, cellulose and pectin. The majority of soybean carbohydrates can be classed as belonging to dietary fibre. According to solubility in water, total dietary fibre can be categorized into two groups, such as soluble and insoluble dietary fibre. Both dietary fibres have been known to play different physiological roles in human health (Vasanthan *et al.*, 2002). Soluble dietary fibre is more effective than insoluble dietary fibre in many healthy aspects. Therefore, preparation of soluble dietary fibre is especially important. Soybean residue is a good dietary fibre resource (Bourquin *et al.*, 1996).

2.6 Antinutritional factors in cereals and soybean

Cereals are rich in minerals but the bioavailabilities of these minerals are usually low due to the presence of antinutritional factors such as phytic acid, trypsin inhibitors and polyphenols. The phytic acids are a major antinutritional factor because it is found in most of the cereals and have strong ability to complex multi-charged metals ions, particularly zinc, calcium and iron (Cheryan and Rackis, 1980). Phytics acid combines with these elements to form insoluble salts called phytate, which are not absorbed by body thereby reducing bioavailability of these elements for human body utilization (Cheryan and Rackis, 1980). Tannin is the other antinutritional factor present in cereals which can bind precipitate protein and decreased its digestibility. Trypsin inhibitors which can impair protein digestibility which have been isolated in pear millet and rye although are normally deactivated by heating (Bender and Bender, 1999). Generally,

tannins inhibit digestibility of protein and phytic acid reduces bioavailability of some essential minerals (Duhan *et al.*, 1989).

Legume such as soybean also contains several antinutritional factors. Protease inhibitors, Kunitz-typsin inhibitor (KTI) and Bowman-Birk inhibitor, Lectins are the major antinutritional factors present in soybean seed. Protease inhibitors represent 6% of protein present in soybean seed. Approximately, 80% of trypsin inhibition is caused by KTI, which strongly inhibits trypsin and therefore reduces food intake by diminishing theirs digestion and absorption (de-Carvalho *et al.*, 1998). Phytic acid is other antinutritional factor present in soybean seeds and products to extent of 1-1.5% of dry matter. It is able to chelate minerals elements, such as zinc, magnesium, iron, calcium and potassium and makes these elements longer absorbed from intestines. About two thirds of total phosphorus from soybean seed is bound to phytic acid (Nelson *et al.*, 1968).

Beany flavor is one of the major unpleasant characteristics and limiting use of conventionally produced soybean flours due to the antinutritional factors. Antinutritional compounds are responsible for bitterness and beany taste of raw soybean. Such chemicals include trypsin inhibitors; which protect the action to breakdown of protein by trypsin; haemaglutinins, which cause agglutination of red blood cells, phosphatidylcholine, which produces an unpleasant flavor and bitterness and raffinose which cause flatulence (Muhsin, 2009).

2.6.1 Phytic acid

Phytic acid and phytate refer to free acid and salt respectively. In the literature, the terms phytate and phytic acid have been used interchangeably (Choi *et al.*, 2001). Approximately 75-85% of total phosphorus in plant seeds is in the form of phytate (Kara *et al.*, 1985) which is the main storage form of phosphorus in cereals, legumes and other plant tissues. Phytic acid or abbreviated IP6 (myo-inositol 1,2,3,4,5,6 hexakis (dihydrogen phosphate)) consists of myo-inositol esterified with six phosphoric acid groups.

2.6.1.1 Chemical structure and properties of phytic acid

Structure of phytic acid has been an intensively discussed subject in a society for many years during twentieth century (Reddy *et al.*, 1982). Phytic acid has two different structures containing 12 acid hydrogen's $C_6H_{18}O_{24}P_6$ that are suggested by Anderson (1914) and Neuberg (1909) structure containing $C_6H_{24}O_{27}P_6$ with 18 acid hydrogen's and, are the most accepted. The structure suggested by Neuberg had three P-O-P linkages between pairs of adjacent phosphate groups (tripyrophosphale) while in the structure suggested by Anderson, the phosphate groups

were not linked. Several studies such as titration of sodium phytate with metal ions, nuclear magnetic resonance (NMR), elemental analysis and X-ray crystallography support each of these structures and have led to conclusion that the structure suggested by Anderson is the correct form present in plant materials (Figure 2.2) (Reddy *et al.*, 1982).

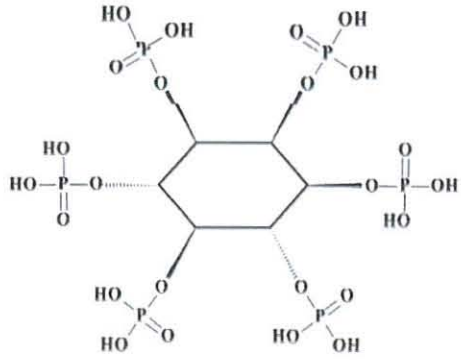


Figure 2.2: Structure of phytic acid suggested by Anderson (1914).

Phytic acid is negatively charged and has potential to bind metals strongly because of strong chelating effect or other positively charged functional groups of molecules at pH values under neutral and alkaline conditions present in foods (Cosgrove and Irving, 1980).When phosphate groups are removed from inositol hexakis phosphate (which leads to fewer phosphate groups than in IP6), the mineral binding strength becomes progressively lower and the solubility increases (Harland and Morris, 1995).

On the other hand, IP6-protein complexes are formed under acidic conditions where most of proteins originating from plants are positively charged (Reddy, 1989). IP6-metal ion-protein complexes have also been suggested to be formed under neutral and slightly alkaline conditions. Generally, phytic acid can form complexes with protein, starch, minerals and trace elements and can thereby affect theirs availability. Nutritionally most important are the interactions of phytic acid with minerals and trace elements, such as iron, zinc, calcium and copper resulting in complexes as has been observed in vitro studies (Vohra *et al.*, 1965). However, extrapolations from in vitro studies in human digestive tract are difficult to make and influence of phytic acid on availability of minerals and trace elements needs to be investigated in human absorption studies.

Complementary foods are first solid foods which are often based on cereals, combined with milk or legumes in countries were milk is not sufficient supply to improve nutritional quality and quantity of protein component. Complementary foods based on cereals and legumes often contain significant amounts of phytic acid that has potentially impairing bioavailability of mineral and trace element. The availability of minerals and trace elements is specially concern in infant

nutrition. Iron deficiency is a major problem mainly affecting infants and children as well as women of childbearing age and is often due to high phytic acid in diets. Similarly zinc is particularly importance as iron deficiency during early life which can reduce growth and immune response (Prasad *et al.*, 1963).

2.6.1.2 Interactions of phytic acid with minerals

The interaction between phytic acid/inositol phosphates and minerals display the existence of an inverse relationship between bioavailability of minerals and inositol phosphates, although there are substantial differences in individual behavior of each mineral element. Solubility studies were often used to investigate the interaction of phytic acid with metals ions since precipitation of phytates could be used as an indication of binding. The stability and solubility of various metals-phytate complexes were determined by measuring a drop of pH (Cheryan and Rackis, 1980).

The replacement of acidic protons by metal ions and shift of phytate ionization equilibrium cause the pH to drop. The magnitude of pH drop indicates the complexing tendency and is a qualitative measure of stability. Based on pH-drop method, zinc formed the most stable complexe with phytic acid followed by copper, nickel, cobalt, manganese, calcium, and iron in decreasing order of stability (Cheryan and Rackis, 1980). The interaction of phytic acid with minerals and other nutrients is depend on pH (Reddy and Sathe, 2001) and the degree of protonation of phosphate groups is a function of pH (Nolan *et al.*, 1987). The molecule works in a wide region of pH as a highly negatively-charged ion, so its presence in food has influence bioavailability of mineral monovalent, divalent and trivalent ions, such as Zn^{2+}, Fe^{2+}/Fe^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} (Lönnerdal *et al.*, 1989) these complexes are more soluble at acid or low pH and insoluble at high or basic pH (Torre *et al.*, 1991).

2.6.1.2.1 Interactions of phytic acid on iron availability

Dietary factors such as phytic acid and polyphenols are affect availability of non-heme iron either positively or negatively (Hurrell, 1997). Many studies performed both in vitro and in vivo, in animals or humans have been conducted to investigate the influence of phytic acid on iron availability (McCance *et al.*, 1943). Different traditional processing methods are available to reduce phytic acid during food preparation had a positive effect on iron availability. Traditional technologies such as soaking and fermentation of cereals grains and starch to reduce phytate content have been shown to improve bioavailability of iron (Ferguson *et al.*, 1993).

The degradation of phytic acid in cereals bran by endogenous phytase almost completely overcame the inhibiting effect on iron availability in adults, thus showing that phytic acid is the

main inhibitory factor in bran (Hallberg *et al.*, 1987). Phytic acid degradation by microbial phytase in soy-protein isolate increased iron availability in adults fed a liquid formula meal (Hurrell *et al.*, 1992). A marked increase the availability of iron was also observed in infants, when infant formula with phytic acid free soy-protein isolate was fed (Davidsson *et al.*, 1994).

2.6.1.2.2 Interactions of phytic acid on zinc availability

Good sources of dietary zinc are meat and meat products and whole grain cereals (Heseker, 1999). The availability of zinc is higher from animal foods than from plant foods. Fractional zinc available in humans can vary considerably from circa 5 to 50%, depend on composition of diet (Fairweather-Tait and Hurrell, 1996). For establishment of zinc requirements, mean fractional absorption of 20% was assumed (Allowances, 1989). However higher fractional availability of (circa 30%) could be expected if zinc intake mainly derives from animal products (Rimbach *et al.*, 1996). Fractional zinc absorption varied from 8 to 38% from composite meals and from 8 to 27% from different cereals (Sandström *et al.*, 1980). Zinc availability was found to be <15% from meals with high contents of phytic acid (Rossander *et al.,* 1992). Phytic acid may interact with zinc which is leading to reduction the availability of zinc.

Disappointingly, zinc does not consistently improve linear growth in intervention studies of toddlers and children. This has been attributed to the fact that zinc intervention may occur at the wrong ages, missing the 6 to 18 month age interval, in which postnatal cell division and growth are most rapid; thus, zinc is given too late. Zinc does play a role in increase of muscle mass in children (Shrimpton *et al.*, 2005). Food fortification with zinc has not included zinc to any great extent to date in developing countries (Ferguson *et al.*, 1993). The agricultural sector has been conducted breeding research and development and has produced high zinc maize and also low phytate maize. To date, the adoption and dissemination of these maize varieties have been limited.

Based on data from animal studies, molar ratios of phytic acid-zinc (Oberleas and Harland, 1981) and molar ratios of calcium x phytic acid-zinc (Davies *et al.*, 1985) have been proposed to estimate the inhibiting effect of phytic acid on zinc absorption. Ellis *et al.* (1987) suggested ratios phytic acid-zinc of >10:1 and calcium x phytic acid-zinc >200:1(mM) to negatively influence zinc availability. Most omnivorous diets were found to be below these critical ratios, while vegetarian diets were above. The molar ratios have been used to estimate zinc bioavailability from different food sources in humans (Fitzgerald *et al.*, 1993). However, Wise (1995) doubted the predictive use of these ratios, derived from animal studies, for humans.

2.6.1.2.3 Interactions of phytic acid on calcium availability

Phytic acid may interact with calcium which is leading to a reduction of calcium availability. Animal and human studies have been reviewed by Reddy (1989). Morris and Ellis (1985) suggested that diets with a molar ratio of phytic acid: calcium > 0.2:1 might lead to calcium deficiency in humans. Calcium availability from soybean with high phytic acid content was significantly lower (31%) than from soybeans with low phytic acid content (41%) when measured in women, using intrinsic labeling with ^{45}Ca (Heaney *et al.*, 1991). Weaver *et al.*(1991) investigated that leavening of products based on wheat flour improved calcium availability. However, wheat products, except bran cereal, did not have a negative effect on calcium availability, which was even better than from milk with similar calcium content.

2.6.1.3 Interactions of phytic acid with protein and starch

pH is the factor that are determine interactions between phytic acid and proteins. When the value of pH low or below isoelectric point, the amino terminal group of protein is positively charged and can bind directly to negatively charge of phosphate group and form binary protein-phytate complexs. They are insoluble complexes that dissolved only at low pH . Such complexes formations might affect the protein structure that can hamper enzymatic activity, protein stability and protein digestibility (Kumar *et al.*, 2010). When pH>6 (above the isoelectric point) phytic acid and proteins are negatively charged and theirs interactions are somewhat unclear. It has been suggested that multivalent cations are bound between negatively charged phosphate group of phytic acid molecule and negatively charged carboxyl group of protein, resulting in a ternary phytic acid-cation-protein complex. At even higher (pH>10) it was suggested that ternary complex dissociates and phytic acid becomes insoluble, while unbound protein remains in solution (Cheryan and Rackis, 1980). Thompson (1987) suggested that other ternary complexes, for example phytic acid-protein-carbohydrate (starch) complexes may form through protein associated with starch (Figure 2.3). Starch can also bind to phytic acid by the formation of hydrogen bonds.

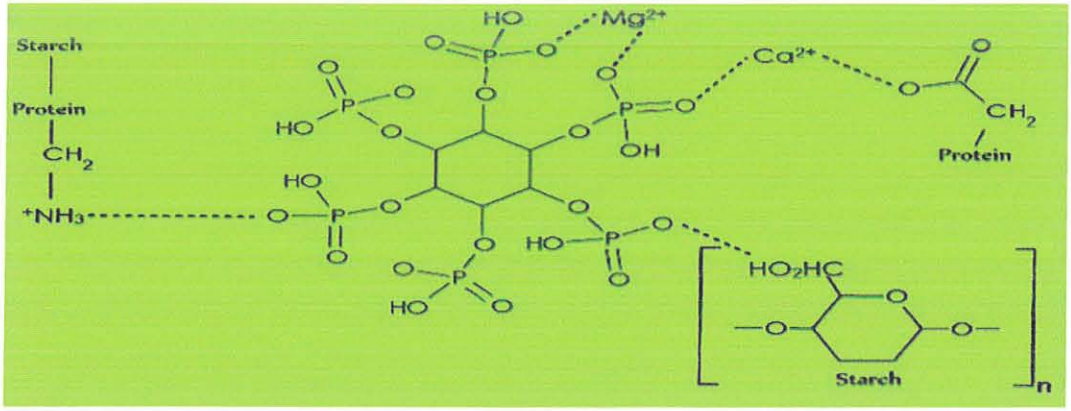


Figure 2.3: Phytic acid interaction with minerals, protein and starch (Thompson, 1987).

2.6.1.4 Applications of phytic acid in food

The ability of phytic acid to chelate metal ions over a broad pH range makes it useful in food industry. In food industry phytic acid can serve as a preservative or antioxidant by formation of free radicals (OH) which is suppressed iron-catalyzed oxidative reactions. Phytic acid can prevent rancidity of food contain lipid and can stabilize natural and artificial coloring agents. At present time phytic acid is sold as a nutrition supplement for athletes in the USA. Distributors claim that daily intakes of 0.75 to 1.50g help to improve the building of bone tissue increase oxygen transport capacity of red blood cells and accelerate transportation of creatine and other nutrients to muscle cells. However, these effects have not been display in humans and orally administered phytic acid is not expected to be absorbed (Graf, 1983)

Approximately adults consume 1g of inositol per day from both animal and plant sources (Harland and Morris, 1995).This value could be as high as 4.5g/day depending on amount of plant-derived foods; an average intake of phytate is 2-2.6g/day for vegetarian diets and inhabitants of rural areas in developing countries (Greiner and Konietzny, 2006). An increased dietary consumption of cereal fibres, legumes and soy protein leads to a higher intake of phytate (Dvořáková, 1998).

The phytase was used either as a powder or in immobilized form (Khare *et al.*, 1994) to prepare phytate-free soybean milk. Some suggestions for reducing phytate in foods and producing low phytate in cereals and soybean enhancing levels of seed phytase by gene transfer, eating more geminating seeds, using yeasts, lactic acid bacteria and other microorganisms producing phytase for bread making and fermented foods, and employing non-enzymatic hydrolysis of phytate efficiently during food processing (Greiner and Konietzny, 2006).

Degradation of inositol hexakis- and pentakisphosphates was essential in order to improve bioavailability of minerals and trace elements (Sandberg and Andersson, 1988). However, positive effects of phytate and its lower phosphate derivatives for health should be considered in diet of inhabitants in developed countries. Intake of phytase or low phytic acid ingredients to food producing animals does not likely causes any health problems since animals live for a relatively short period of time and do not normally receive high levels of dietary iron (Sands *et al.*, 2003). However, low phytic acid diets may have potentially effects on human health, in particular for people with high iron stores caused by elevated dietary intakes of highly available iron from animal products and fruit which greatly enhance availability of non-heme iron (Fleming *et al.*, 2002). The presence of undigested phytate in colon may prevent from development of colonic carcinoma (Iqbal *et al.*, 1994).

Consumption of dietary phytate was suggested to prevent kidney stone formation and to protect atheriosclerosis and coronary heart disease (Jariwalla *et al.*, 1990) as well as protection from variety of cancers (Vucenik and Shamsuddin, 2003). The levels of phytate and its dephosphorylation products in urine, plasma and other biological fluids are fluctuating with ingestion or deprivation of phytate in human food (Grases *et al.*, 2001). Therefore, reduction in phytate intake in developed compared to developing countries might be a factor responsible for increase in diseases typical for Western societies such as diabetes mellitus, atheriosclerosis, cancer, renal lithiasis and coronary heart diseases.

2.6.2 Tannins

Tannins are naturally occurring complex group of polyphenolic compounds found in a wide range of plant species that influence the nutritive value of legumes (Haslam, 1989). Tannins are widely distributed throughout the plant kingdom, particularly trees, shrubs and herbaceous leguminous plants (McLeod, 1974). However, several soluble phenolics that have similar structural and chemical properties to tannins that do not precipitate proteins. Tannins exist in mixtures with many other classes of plant phenolic compounds. Inconsistent relationships between the definition of tannins and the analysis of mixtures of phenolic compounds complicate research on the nutritional toxicology of tannins in forage legumes (Haslam, 1989).

2.6.2.1 Chemical structure and properties of tannins

Traditionally tannins are classified into two groups: Hydrolysable tannins and condensed tannins. The hydrolysable tannins (HT) are made up of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids primarily gallic and hexahydroxydiphenic acid

(McLeod, 1974). Hydrolysable tannins are further classified according to the products of hydrolysis; gallotannins yield gallic acid and glucose and ellagitannins yield ellagic acid and glucose (Haslam, 1989). Several tannins can be fractionated hydrolytically into their components, for example by treatment with hot water or with tannases which are classified as hydrolysable tannins (Ree, 2001).

Hydrolysable tannins are derivatives of gallic acid (3,4,5-trihydroxyl benzoic acid). Galic acid is esterified to a core polyol and the galloyl groups may be furthermore esterified or oxidatively crosslinked to yield more complex hydrolysable tannins. Gallotannins are simplest hydrolysable tannins which are simple polygalloyl esters of glucose. The prototypical gallotannin is pentagalloyl glucose (β-1,2,3,4,6-Pentagalloyl-O-D-Glucopyranose).Tannic acid, commercially available gallotannin is the most suitable starting point for purification of gallotannins. Commercial tannic acid can be fractionated chromatographically to yield specific galloyl esters or can be methanolyzed to yield homogeneous pentagalloy glucose (Hartzfeld *et al.,* 2002).

Non-hydrolysable oligomeric and polymeric proanthocyanidins were classified as condensed tannins (Ree, 2001). The condensed tannins (CT) or proanthocyanidins are non-branched polymers of flavonoids units (flavan-3-ol, flavan-3,4-diol), and usually have a higher molecular weight than the HT (1000-20000 Da compared to 500-3000 Da) (McLeod, 1974). The most widely studied tannins are based on the flavan-3-ols (-)-epicatechin and (+) - catechin.

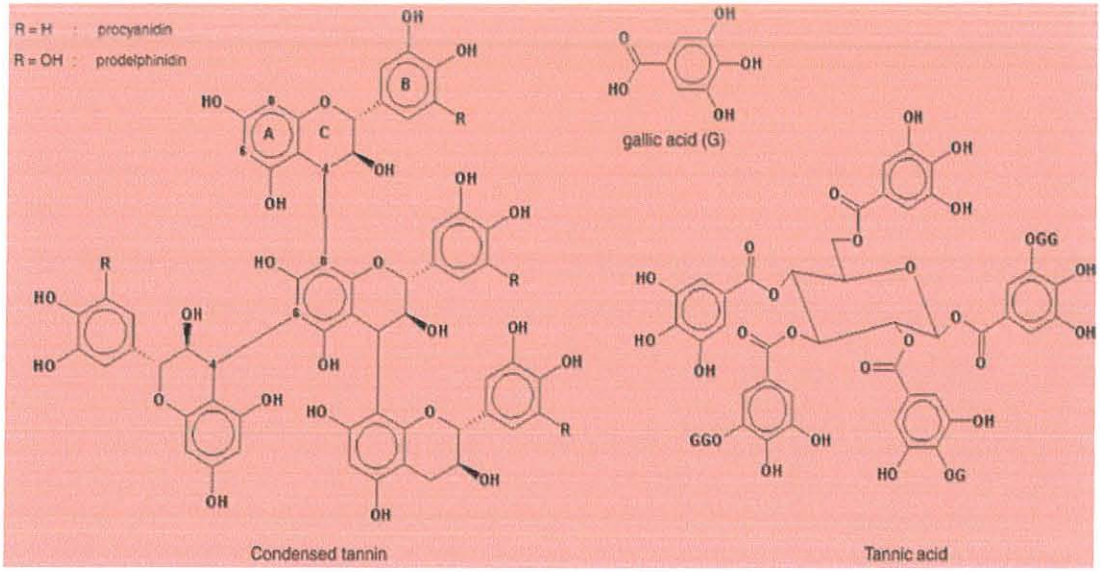


Figure 2.4: Possible molecular arrangements of an oligomer of condensed tannin and tannic acid (Kaal *et al.*, 2007).

2.6.2.2 Interactions of tannins with proteins and starch

Tannins are phenolic compound that have high molecular weight containing sufficient phenolic hydroxyls and other suitable groups (i.e., carboxyls) to form effectively strong complexes with protein, starch and cellulose under particular environmental conditions (Horvath, 1981). The ability of tannins to form strong complexes with proteins is the most important aspect of their nutritional and toxicological effects. The strength of tannins-protein complexes depends on properties of tannin and protein (molecular weight, isoelectric point and compatibility of binding sites) (Hagerman and Butler, 1991).

Tannins have a large number of free phenolic hydroxyl groups that form strong hydrogen bonds with proteins as well as carbohydrates (Haslam, 1989). In addition, tannins form covalent bonds with proteins through oxidative polymerization reactions as a result of heating, exposure to UV radiation, and the action of polyphenol oxidase (Frutos *et al.*, 2004). Tannin has ability to precipitate certain proteins which combined with digestive enzymes thereby making them unavailable for digestion (Abara, 2003). Tannins also form insoluble complexes with carbohydrates and lipids leading to a reduction in digestibility of these nutrients (Binita and Khetarpaul, 1997).

2.6.2.3 Interactions of tannins with minerals

The bioavailability of a mineral is defined as the proportion of the total amount of the mineral in a food that is actually obtained to ensure normal metabolic functions. In cereal- legume based foods the availability of minerals such as zinc and iron for intake is limited due to the presence of tannins in grains. For example, bran of cereals grain are particularly rich in tannins and minerals because of this reason the bioavailability of iron and zinc in bran may be minimized due to the presence of complexes formed between these compounds (Lestienne *et al.*, 2005). Tannins and certain insoluble fibres are the main compounds that can interact with iron and/ or zinc ions (Gillooly *et al.*, 1984).

Dietary iron or nonheme irons from plant origin are essential for the world population (Morck and Cook, 1981). Poor intake of iron is an important problem since the bioavailability of non-heme iron is influenced by other dietary components. In the past decade, tannins have been identified as potent inhibitors of non-heme iron intake. The effects of tea on iron intake were first reported by Disler *et al.* (1975). Phenolics can influence availability or activity of metal ions by chelating the metal. It is widely believed that tannin-chelated metal ions are not bioavailable. For example, consumption of large quantities of tea or other tannin-rich foods is sometimes associated with deficiency diseases such as anemia. As compared to water, drinking tea minimized the intake of ferric chloride by 16% and ferrous chloride with ascorbic acid by 20% (Disler *et al.*, 1975).

The presence of tannic acid in a food has been shown to reduce iron intake. The inhibition of iron intake by tannic acid is likely due to galloyl groups which have a direct chemical binding effect particularly on ferric iron and also presumably through the formation of chelates. Polyphenols are thought to act through the formation of complexes between hydroxyl groups of phenolic compounds and iron molecules rendering iron unavailable for intake (Graf, 1983).In developing countries due to low access to animal products such as meat, fish and eggs the availability of high intakes of zinc are cereals and legumes which are the main dietary sources of zinc.

The cereal represents an important proportion of dietary intake for millions of people. Unfortunately, the zinc in cereal based foods is poorly bioavailable due to factors that reduce their intestinal absorption, resulting in high rates of zinc deficiency particularly in infants and children (Sandstead, 2000). Over the years, many in vivo (Larsson *et al.*, 1996) or in vitro (Olivares *et al.*, 2001) studies have reported the negative effects of tannins on zinc bioavailability or in vitro

availability. The study shows that the effects of tannins on zinc availability are highly dependent on food matrix.

2.6.2.4 Applications of tannins in food

Tannins are one of the major groups of antioxidant polyphenols found in food and beverages which have attracted a lot of attention in recent years because of theirs beneficial to human health. Food contained in low tannins are multifunctional to human health because of their meaningful antioxidant properties (Dixon *et al.*, 2005). These antioxidant compounds fall into three major categories such as tannins, phenolic acids and flavonoids. Among these, tannins account for about 19% of total dietary antioxidant capacity (Floegel *et al.*, 2010). Condensed tannins are widely distributed in fruits, vegetables, red wine and certain food grains such as sorghum, finger millets and legumes. Beneficial effects of tannins from foods rich include immune modulatory, anti-inflammatory, antimutagenic, vasodilating, antithrombotic effects and UV-protective functions (Dixon *et al.*, 2005).

Tannins are widespread throughout plant kingdom with diverse biological and biochemical functions such as protection against predation from herbivorous animals and pathogenic attack from bacteria and fungi (Xie *et al.*, 2003). Interestingly, tannins are also found in grains, such as sorghum (Sorghum bicolor) with a pigmented testa layer, some finger millets and barley (Dykes and Rooney, 2007). Tannins are the most uniquely important phytochemical components of sorghum since they possess properties that produce obvious and significant effects in animals, and have also been associated with various positive and negative impacts on human health. Tannins from sorghum show powerful antioxidant activity in vitro. We also found that tannin (brown) sorghums had antioxidant activities higher than when compare with most non-tannin sorghums (Hagerman *et al.*, 1998).

Available epidemiological evidence suggests that sorghum consumption reduces the risk of certain types of cancer in humans compared to other cereals. The high concentration of phytochemicals in sorghum may be partly responsible. Tannins found in sorghums are widely reported to reduce caloric availability and hence weight gain in animals. This property is potentially useful in helping minimize obesity in humans. Sorghum phytochemicals also promote cardiovascular health in animals. Such properties have not been reported in human and require investigation, since cardiovascular disease is currently the leading killer in the developed world (Awika and Rooney, 2004).

2.7 Processing methods for reduction of antinutritional factors

Several traditional food processing methods and treatment conditions are available to minimize ANFs present both in cereals and legumes seeds. The effects of processing methods are enhances bioavailability of minerals and improve flavor and increase overall acceptance of plant-based diets through reduction of antinutrients (Monari *et al.*, 1993).

2.7.1 Fermentation

Traditional methods such as fermentation improve the nutrient quality of foods and, are associated with many chemical changes that enhance nutritional quality and organoleptic properties, contents of free sugars and vitamins, as well as bioavailability of minerals (Zamora and Fields, 1979) and results breakdown of some anti-nutritional endogenous compounds. Fermentation also improves protein quality and digestibility, vitamins B content, and microbiological safety and keeping quality. Low molecular-weight organic acids (e.g. citric, malic and lactic acid) are produced during fermentation that have potential to enhance iron and zinc absorption from cereals or legume flour (Teucher *et al.*, 2004) reduced.

Table 2.6: Effect of fermentation on food and potential health benefits

Effect on food	Potential health benefits
Break down of starch by amylases	Reduces bulk and increases energy intake
Reduction of phytic acid	Improved absorption of minerals and protein
Decrease in pH	Improved absorption of minerals and food safety
Increase in lactic acid bacteria	Better food safety and potential probiotic effects
Synthesis of B vitamins	Better vitamin B status

Source: (Bekele, 2011).

Fermentation causes changes in food quality including pleasant flavors, aromas textures, appearance, nutrition and safety. The benefits of fermentation may include improvement in palatability and acceptability by developing improved flavor and texture (Parveen and Hafiz, 2003). The changes occurring during fermentation process are mainly due to enzymatic activity exerted by microorganisms and/ or indigenous enzymes in the grain (Svanberg and Lorri, 1997). During cereal fermentations, several volatile compounds are formed, which contribute to a complex blend of flavors in the products. The presence of aroma representatives; diacetyl, acetic acid and butyric acid make fermented cereal based products more appetizing (Katongole, 2008).

The microorganisms involved in natural fermentation of cereals are essentially the surface flora of the seeds (Svanberg and Lorri, 1997). In general, natural fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly and oligosaccharides. Certain essential amino acids are synthesized and the availability of B group vitamins is improved. Fermentation also provides optimum pH conditions for enzymatic degradation of phytates which is present in cereals in the form of complexes with polyvalent cations such as iron, zinc, calcium, magnesium and proteins. Such phytate reduction may increase the amount of soluble iron, zinc and calcium (Katongole, 2008).

2.7.2 Soaking

Different food processes such as soaking cereals and most legumes in water can result in passive diffusion of water soluble Na, K, or Mg-phytate, which can then be removed by decanting the water. The extent of the phytate reduction depends on the species, pH and length and condition of soaking. A simple soaking procedure appropriate for rural subsistence households has been developed that can reportedly reduce the phytate content of unrefined soybean. Several recent in vivo isotope studies in adults and infants have reported improvements in absorption of iron, zinc, and calcium in cereals-based foods prepared with reduced phytate content. Some oxalates and polyphenols that inhibit calcium and iron absorption, respectively, may also be lost by soaking (Ologhobo and Babatunde, 1984).

2.7.3 Dehulling

Dehulling is one of processing methods and traditional treatments to remove soybean hulls (coat) that contain unwanted substances such as tannins and high-lignin fibres present in the hull. The hulls, therefore, should be removed to reduce off-flavor. In addition, dehulling improves the nutritional quality of soybeans to various extents. The preparation of beans for processing is important to achieve good hull removal. This step is critical for manufacture of high-protein dehulled soy meal. However, the complete separation of the hulls from the meal of the soybean during processing is unlikely. Dehulled soybean is produced by wet methods which, involves soaking the whole bean in water for some times and removing the hulls manually and drying the cotyledons (Hotz and Gibson, 2007).

2.7.4 Roasting

Roasting methods involve the treatment of soybean with a temperature varying between 110 to 170°C (Bekele, 2011) to improve the taste and edibility of soybeans. In addition, roasting is important to reduce the anti-nutritional factors. In any case, a uniform treatment must be sought, thus avoiding a situation in which the core of some of the particles remains raw whilst the outer layer of others has been over processed. It is important to divide the different beans into size categories before roasting in order to prevent the overheating of the smallest ones. Roasting has a significant impact on the overall quality of legumes and the final product (Mridula *et al.*, 2007).

CHAPTER THREE

Materials and methods

3.1 Raw materials collection, sample preparation and storage

Sorghum variety (Gobyie), Maize variety (Melkassa 4) and Soybean variety (ETV) were collected from the Ethiopian Seeds Enterprise (ESE), Arsi Basic Seeds Storage and Preparation Center, Asella, Ethiopia. Sample preparation was done at Addis Ababa University, Nutrition Laboratory, Center for Food Science and Nutrition and Ethiopian Health and Nutrition Research Institute.

Preparation of fermented sorghum flour

Seed grains were sorted and cleaned manually to remove broken seeds, dust and other extraneous materials. Whole sorghum grain was soaked in water (1:3) at room temperature for 48hrs. After soaking the grains were dried in an oven for 12hrs and milled into fine flour with a hammer mill and sieved with 0.425 mesh size screen to obtain the flour.

Natural fermentation was carried out by using microorganisms naturally present on the grain surface which is performed by mixing sample with distilled water (1:3 w/v). Three hundred gram of sorghum flour was mixed with nine hundred ml of distilled water in a cleaned plastic bucket and allowed fermentation at room temperature for 72hrs. The slurries were transferred into aluminum foil, then dried using freeze drier for 10hrs. Fermented and dried sorghum products were further milled to fine flour using hammer mill and packed in polyethylene bag and stored at ambient temperature for subsequent analysis (Adebayo-Oyetoro *et al.*, 2012). The show diagram is shown in the Figure 3.1.

Preparation of fermented maize flour

Maize grains were sorted and cleaned manually to remove broken seeds, dust and other extraneous materials. Whole maize grain was soaked in water (1:3) at room temperature for 24hrs. After soaking, the grains were dried in an oven for 12hrs and milled into fine flour with a hammer mill and sieved with 0.425 mesh size screen to obtain the flour. Natural fermentation was carried out by using microorganisms naturally present on the grain surface which is performed by mixing flours with distilled water (1:3w/v). Three hundred gram of maize flour was mixed in nine hundred ml of distilled water in a cleaned plastic bucket to allow fermentation at room temperature for 72hrs. The slurries were transferred into aluminum foil, then dried using freeze drier for 10hrs. Fermented and dried maize dough were further milled to fine flour using hammer

mill and packed in polyethylene bag and stored at ambient temperature for subsequent analysis (Amankwah *et al*., 2009b). The show diagram is shown in the Figure 3.1.

Preparation of soybean flour

Soybeans were sorted for stones, rot and other physical defects. The beans were then washed and soaked in distilled water 1:5w/v for 15hrs according to Yimer (2008). The soaked beans were then placed in a sieve and allowed to drain. The drained seeds were blanched in boiling water for about 20min (Bekele, 2011). This step is called blanching. Blanching and dehulling easier to inactivate enzymes activities. The hulls were removed manually, then after removing the hulls it was washed repeatedly using distilled water. The dehulled beans were then dried using tray dryer up to moisture content of 11-13%. The dehulled beans were roasted using an electric oven for 8min at a temperature of 110-130^{0}C until it gets brown to further reduce anti-nutritive factors and improve the flavor of the final products. The roasted soybeans were milled with hammer mill into flour and sieved through 0.425 mesh size screen. The shown diagram is shown in the Figure 3.1

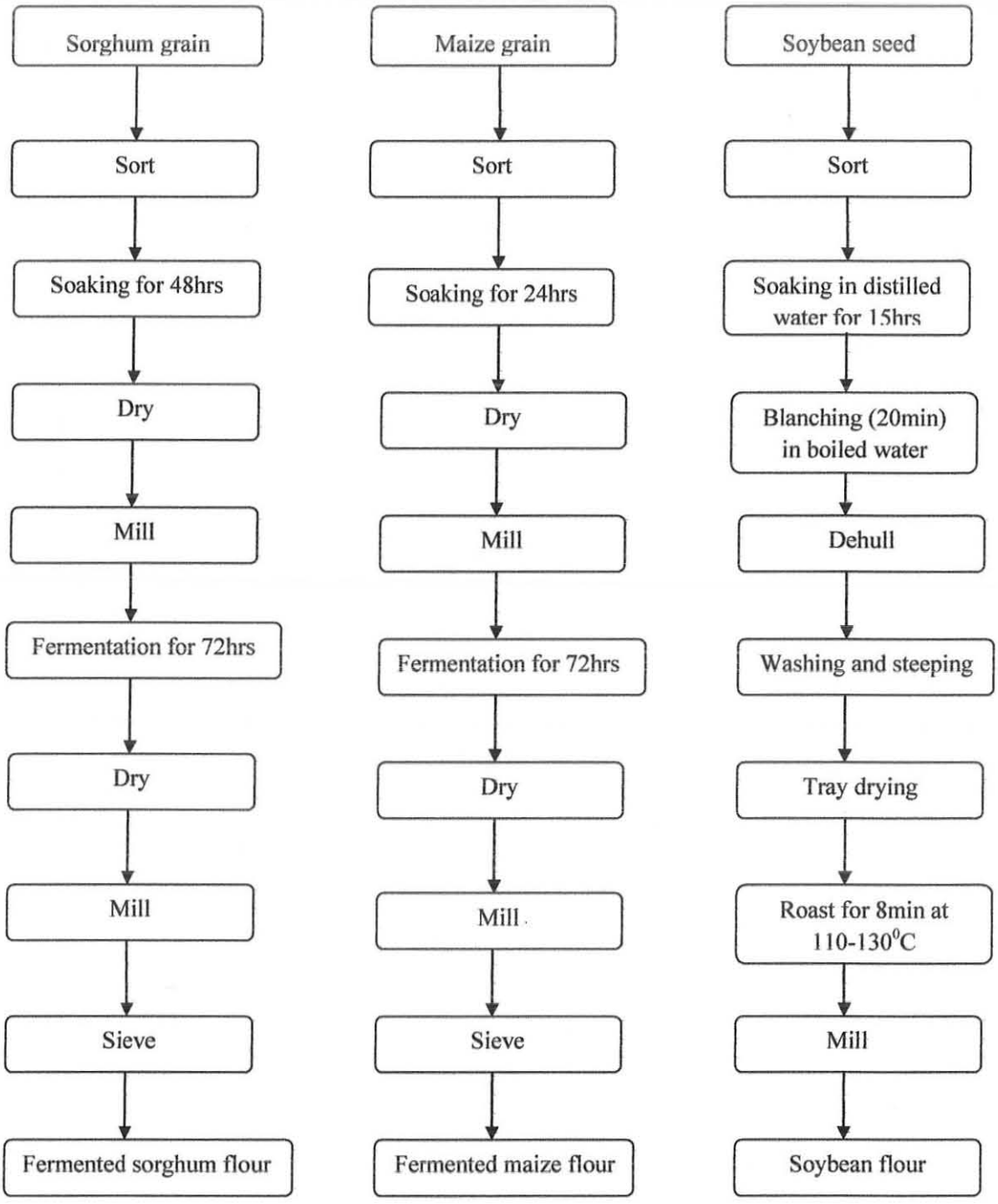


Fig 3.1: Flow chart for the processing of sorghum, maize and soybean flours (Adebayo-Oyetoro *et al.*, 2012; Amankwah *et al.*, 2009b; Yimer, 2008).

3.2 Formulation of complementary foods

In order to formulate complementary diets, the material balance method requires the use of proximate values of the raw materials (Amankwah *et al.*, 2009a).Therefore, the primary criteria is to select the components rich in providing protein and energy requirements, the next target is to know the proximate values of the raw materials that are going to be blended. They are required as an input for materials balance. Of these compositions commonly used for formulation are protein, carbohydrates and fat that provide body with energy.

The output components used in the materials balance methods was from FAO or WHO standards based on the target age. Therefore, the materials balance methods was used to target reference to protein requirement of infants 18% protein, 59% carbohydrate (Amankwah *et al.*, 2009a) and minimum energy value of 380kcal/100g in dry matters according to WFP requirement specifications in the complementary blend formulation for particularly age group of 6 to 24 months. In case of this research, carbohydrate and protein composition of sorghum, maize and soybean flours were used based on the age group of infants for the complementary foods. Therefore material balance method was used to achieve the control blend formulation of 70% maize and sorghum flours to 30% soybean flour (Figure 3.2).

Targeting the blends to have 18% protein and 59% carbohydrate (Bekele, 2011).

Component balance on protein

Protein of cereal* cereal + Protein of soybean* Soybean = 18 ..... (1)

Component balance on carbohydrate

Carbohydrate of cereal * cereal + carbohydrate of soybean*Soy = 59 ..... (2)

Total balance

Cereal + Soybean = 100 ..... (3)

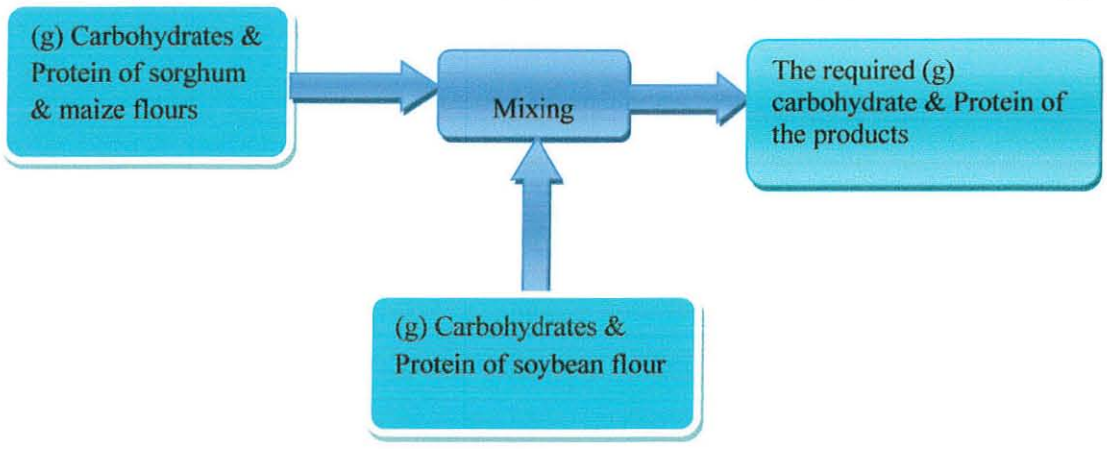


Figure 3.2 Formulation of complementary foods (Bekele, 2011).

Table 3.1: Mixing formulation

Sample code	Formulation name	Mixing ratio (% w/w)
Diet 1	Unfermented sorghum alone (control)	100
Diet 2	Fermented sorghum alone	100
Diet 3	Unfermented sorghum/soybean	70:30
Diet 4	Fermented sorghum/soybean	70:30
Diet 5	Unfermented maize alone (control)	100
Diet 6	Fermented maize alone	100
Diet 7	Unfermented maize/soybean	70:30
Diet 8	Fermented maize/soybean	70:30
Diet 9	Soybean alone	100

3.3 Analytical methods

3.3.1 Determination of proximate composition

3.3.1.1 Determination of moisture content

Moisture of each sample was determined according to AOAC (2000) using the official methods 925.09. Empty drying dishes (made of porcelain) were dried using a drying oven (Germany, Memmert) for 1hrs at 100^{0}C. The dishes were cooled for 30 minute in desiccators and weighed using a digital analytical balance to the nearest milligram. About 5g of well prepared fresh samples (in triplicate) were transferred into dried and weighed drying dishes. The dishes and their contents were placed in drying oven and dried for 4hrs at 105^{0}C. The dishes and their contents were cooled in desiccators to room temperature and weighed. The procedure was repeated until a constant weight was recorded (Osborne and Voogt, 1978). Then, the moisture content was estimated by the formula:-

Moisture content (g/100g) = [((weight of fresh sample-weight of dry sample)/ (weight of fresh Sample))]*100.

3.3.1.2 Determination of total ash content

Porcelain dish were placed in a muffle furnace (Carbolite, Aston Lane, Hope, Sheffield, England, UK) for 30 min at 550^{0}C. The dishes were cooled in desiccators for about 30 minutes and weighed to the nearest milligram. About 2.50g of fresh sample in duplicate was placed in dish. Dish was placed on a hot plate under a fume-hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes with sample were

placed inside the muffle furnace at 550^{0}C for 5hrs and cooled in desiccators for 1hrs. The ash was clean and white in appearance. When cooled to room temperature, each dish with ash was reweighed to the nearest milligram. The ash content was determined by Yimer (2008) using the AOAC (2000) official methods 923.03 and applying simple formula:-

$$\text{Total ash content (g/100g)} = [((W_2\text{-}W)/ W_1\text{-}W))*100$$

Where: W= Weight in grams of empty dish, W_1= Weight in grams of the dish plus the dried test materials and W_2= Weight in grams of the dish plus ash

3.3.1.3 Determination of crude protein content

Digestion: Protein content was determined according to AOAC using the official method 979.09 (Yimer, 2008). About 0.5g of each samples were taken in a Tecator tube and 6ml sulfuric acid was added and mixed, and 3.5ml of 30% hydrogen peroxide was added step by step. As soon as the violent reaction had ceased, the tubes were shaken and placed back to the rack. 3g of catalyst mixture (ground 10g of copper sulfate with 150g of potassium sulfate) were added into each tube and exposed to about 370^{0}C in order to allow digestion.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml de-ionized water and the rinses were added into the solution. A 25ml of 40% sodium hydroxide solution was added into the compartment and washed down with a small amount of water, stoppered and the steam switched on. A 100ml solution of the sample was distilled, and then the receiver was lowered so that the tip of the condenser is above the surface of the distillate. The distillation was continued until a total volume of 150ml is collected. The tip was rinsed with a few milliliter of water before the receiver was removed.

Titration: The distillated solution was titrated with 0.1N Hydrochloric acid to a reddish color.

Calculation:

$$\text{Total nitrogen (\%)} = [((V\text{-}V_b)*N*1.4)]/w$$

$$\text{Crude protein content (g/100g)} = \text{Total nitrogen (\%)}*\text{conversion factor}$$

Where: V = Volume of acid consumed to neutralize the sample; V_b = Volume of acid consumed to neutralize the blank; N = normality of the acid; 14 = Eq. wt of nitrogen. w = weight of sample

N.B. 6.25 was conversion factor for maize and sorghum flours whereas 5.71 and 5.98 were conversion factor for soybean and blend flours respectively.

3.3.1.4 Determination of crude fat content

Extraction flasks were dried in a drying oven (Memmert, Germany) at 92^{0}C for 30 minutes, cooled in desiccators (with granular silica gel) for 30 minutes and then weighed. About 2.00g of fresh samples (in duplicate) were added into extraction thimbles, and then covered with about 2cm layer of fat free cotton. The thimbles with sample were placed into a Soxhlet Extraction Chamber (Soxtec, Manual extraction unit, Foss Tecator, Hoganas, Sweden). Cooling water was switched on, and 50ml of diethyl ether was added to the extraction flask through the condenser. The extraction was conducted for 4hrs at 55^{0}C. The extraction flask with fat was removed from the Extraction Chamber and placed in the drying oven at 92^{0}C for about 30 minutes, cooled to room temperature in desiccators for about 30 minutes and reweighed (Tizazu *et al.*, 2009).

Calculation:

$$W = W_2 - W_1$$

$$\text{Crude fat content (g/100g) in fresh sample} = (W*100)/Ws$$

W = weight of fat (g); W_2 = weight of extraction flask after extraction (wt. of flask and fat); W_1 = weight of extraction flask before extraction (wt. of flask); Ws = weight of fresh sample.

3.3.1.5 Determination of crude fibre content

Crude fibre analysis was conducted using the methods of AOAC official methods 962.09 (Yimer, 2008). About 1.5g weighed sample was transferred into a 600ml beaker and about 200ml 1.25% sulfuric acid was added and boiled for 30 minutes. Recording took place a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20ml 28% KOH was added and again boiled gently for further 30 minutes. Subsequently, washing was conducted with 1% sulfuric and NaOH solution. Then, filtered and dried it in the electric oven (memmert 854 Schwabach, West German) at 130°C for 2hrs. Furthermore, it was cooled at room temperature for 30 minutes in a desiccators and weighed, then transferred it to crucible to muffle furnace (GALLENKAMP, Model FSL 340-0100, U.K) for 30 minute ashing at 550°C. Finally, it was cooled again in desiccators and re-weighed. The crude fibre content was determined by using the formula:

$$\text{Crude fibre content (g/100g)} = [(W_1-W_2)*100]/W_S$$

Where: W_1 = weight of (crucible + sample) after drying; W_2 = weight of (crucible + sample) after ashing; W_S = weight of fresh sample

3.3.1.6 Determination of total carbohydrate content

Total carbohydrate content was estimated by the difference in dry weight basis (Ijarotimi and Keshinro, 2012).

Total carbohydrate content (g/100g) = 100 – (Protein (g) + Fat (g) + Ash (g) + Fibre (g))

3.3.1.7 Determination of energy value

Energy value was determined by calculation from fat, carbohydrate and protein contents using Atwater's conversion factors. One gram of carbohydrate was assumed to give 16.72kJ; One gram of fat 37.71 kJ energy and one gram of protein 16.72kJ energy and expressed in calories (Nguyen *et al.,* 2007).

Energy value = (P*16.72) + (F*37.71) + (C*16.7) in kJ/100g of the sample

Where: P = Protein content (g), F = Fat content (g) and C = Carbohydrate content (g)

3.3.2. Mineral analysis

Zinc, iron and calcium were determined using atomic absorption methods Osborne and Voogt (1978). The ash obtained after dry ashing at 525^{0}C was treated with 7ml of 6N of HCl to wet it completely and 7ml of 3N of HCl was added and the dish was heated on the hot plate until the solution just boils. The ash solution was cooled to room temperature in a hood and filtered into a 50ml graduated flask using a filter paper (Whatman 42, 125mm). 5ml of 3N of HCl was added into each crucible dishes and heated until the solution just boils, cooled, and filtered into the flask. The crucible dishes were again washed three times with deionized water; the washings were filtered into the flask. A 2.5ml of 10% lanthanum chloride solution was added into each graduated flask. Then, the solution was cooled and diluted to 50ml with deionized water. A blank which contains 12ml 3N of HCl and deionized water in 50 volumetric flasks was also prepared.

Standard solutions: Four series of working standard metal solutions (Table 3.2) were prepared by appropriate dilution of the metal stock solutions (nitrate of the metal) with deionized water contain 2.4ml 3N of HCl in 10ml volumetric flask. After manipulating the instrument operation procedure, calibration graph (concentration versus absorbance) for each element using the prepared standard solutions was prepared. The sample concentrations were analyzed using Flame Atomic Absorption Spectrophotometer (Varian SpectrAA-20 Plus, Varian Australia Pty., Ltd., and Australia) by aspirating deionized water. Sample blank solution was run with the sample solution.

Table 3.2: Series of working standard solutions for mineral determination

No	Elements	Concentration of standard (µg/ml)
1	Iron	0.00, 2.00, 6.00, 10.00, 12.00
2	Zinc	0.00, 0.60, 1.00,1.40, 1.80
3	Calcium	0.00, 1.00, 1.50, 2.50, 3.00

Metal content (mg/100g) = [(a-b) x V]/10 W

Where, W = Weight of sample in (g), V = Volume of extract (ml), a = Concentration of sample solution (ug/ml) and b = Concentration of blank solution (ug/ml).

3.3.2.1 Mineral Safety Index (MSI)

MSI is a numerical statement of the minimum toxic doses of minerals. The standard MSI for Na, Ca, Mg, Fe, Cu, Zn are 4.8, 10, 15, 6.7, 33 and 33, respectively while the Recommended Infant Intake (RII) are 400, 400, 80, 10, 0.65 and 6, respectively (Oyarekua, 2011). MSI is calculated using formula,

$$\text{Calculated MSI} = \frac{\text{Standard MSI X Value of mineral in sample}}{\text{RII}}$$

3.3.3 Determination of some antinutritional factors

3.3.3.1 Determination of condensed tannin

About 1.0g of fresh sample was weighed in screw cap test tubes (in duplicate). The samples were extracted with 10ml of 1% HCl in methanol for 24hrs at room temperature with a mechanical shaking. After 24hrs shaking, the solution was centrifuged at 1000rpm for 5 minutes. One ml of supernatant was taken and mixed with 5ml of Vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol). D-catechin was used as standard for condensed tannin determination. 40mg of D-catechin was weighed and dissolved in 1000ml of 1% HCl in methanol, which was used as stock solution. Exactly 0, 12, 24, 36, 48, 60 and 1ml of stock solution was taken in test tubes and the volume of each test tube was adjusted to 1.0ml with 1% HCl in methanol. 5ml of Vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of the solutions and the standard solution were measured at 500nm using UV-VIS spectrophotometer by using deionized water as blank, and the calibration curve was constructed from a series of standard solution using SPSS Version 20. Concentration of tannin was read in mg of D-catechin per gm of sample (Maxson and Rooney, 1972).

Calculation:

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

Tannin (mg/100g) = [(absorbance-intercept)/(slope*density*weight of sample)]*10

3.3.3.2 Determination of phytate content

Phytate content was determined by following the method of using Latta and Eskin (1980) as modified by Vaintraub and Lapteva (1988). About 0.0300 mg of dried sample was extracted with 10ml 2.4% HCl for 1hrs at an ambient temperature and centrifuged at 3000rpm for 30 minute. The clear supernatant was used for phytate estimation. A 2ml of wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalicilic acid in water) was added to 3ml of the sample solution (supernatant) and the mixture was mixed on a Vortex (Maxi Maxi II) for 5 seconds. The absorbances of the sample solution were measured at 500nm using UV-VIS spectrophotometer (Bechman DU-64-spectrophotometer).

A series of standard solution were prepared containing 0, 5, 9, 18, 27 and 36 µg/ml of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3ml of standard was added into 15ml of centrifuge tubes with 3ml of water which were used as a blank. A 1ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 20 seconds. The mixture was centrifuge for 10 minutes and the absorbance of the solution (both the sample and standard were measured at 500nm by using deionized water as a blank. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation. The concentration of phytates was calculated using phytic acid standard curve and results were expressed as of phytic acids in mg/100g fresh weight.

Phytic acid (mg/100g) = [(blank absorbance-sample absorbance)-Intercept]*10/Slope*weight of sample*3

3.3.4 Phytate mineral molar ratio calculation

The millimoles of phytic acid and iron were calculated by dividing the milligrams of phytic acid by 660.0 (atomic weight of phytate ion) and the milligrams of 55.8 (atomic weight of iron). The molar ratio was then calculated by dividing millimoles of phytic acid by millimoles of iron. The millimoles zinc was calculated by dividing the milligrams of zinc by 65.4 (atomic weight of zinc). The molar ratio was then calculated by dividing millimoles of phytic acid by millimoles of zinc. Similarly, millimole of calcium was calculated by dividing the milligrams of calcium by 40.0 (atomic weight of calcium). 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 (Bains *et al.*, 2011).

3.3.5 Determination of physico-chemical properties

3.3.5.1 Determination of pH value

The pH of the sample was determined by mixing 10g each sample with 100ml of distilled water. The mixture was left at room temperature for 30min. The pH of the supernatant was then measured by inserted directly into the fermenting dough samples and took readings of values from digital pH meter (Amankwah *et al.*, 2009a). The pH meter was calibrated using pH 4.0 and 7.0 buffers.

3.3.5.2 Determination of total titratable acidity

Total titratable acidity expressed as percentage of lactic acid, was determined by mixing 10g of each sample with 100ml of distilled water and titrating 10ml aliquots with a standard alkali solution of 0.10 N NaOH to 3 drops phenolphthalein endpoint until we got a constant light pink color. The percentage titratable acidity was calculated (Pearson, 1976). Therefore, the titratable acidity was calculated as:

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

Where: V = Volume of 0.1N NaOH used for sample titration; 0.009008 = Factor equivalent in which 1ml of 0.1N NaOH = 0.009008g $C_3H_6O_5$; W= Weight in gram of sample in the mixture (Pearson, 1976).

3.3.5.3 Determination of viscosity

The gruels were prepared in a glass beaker by mixing 10g of flour and 200ml of water. The mixture of water and flour were cooked at 92^0C for 15 minutes. The gruel was placed in a water bath maintained at 45°C (heating temperature) and its viscosity was measured at this temperature. The paste viscosity was measured using a Brookfield Viscometer (Model RVDV-I). The cooked gruel was poured into the viscometer beaker, cooled to 45^0C and viscosity was measured (in centipoises, cP) using spindle number 6, at shear rate of 3 rpm (Mosha and Vicent, 2004).

3.3.6 Determination of functional properties

3.3.6.1 Bulk densities of the flours

Bulk density was determined by the method of Okaka and Potter (1979). A 5g flour sample was put into 100ml measuring cylinder. The cylinder was tapped until there was no further

change in volume. The weight of the tube and its contents was taken and recorded. Bulk density was calculated as weight per unit volume of the sample.

3.3.6.2 Dispersibility of flours

Dispersibility in water which indicates their ability to reconstitute was determined by the methods of Kulkarni *et al.*(1991). 10g of each flour samples were weighed into a 100ml measuring cylinder. Distilled water was added up to 100 ml volume. The sample was vigorously stirred and allowed to settle for 3hrs. The volume of settled particles was recorded and subtracted from 100 to give a difference that is taken as percentage dispersibility.

3.3.6.3 Water absorption capacity (WAC)

The WAC which gives an indication of the amount of water available for gelatinization was determined according to methods of Beuchat (1977). 1g of each samples were measured and mixed with 10ml of distilled water and vortex for 1 min and then centrifuged at 3000 rpm for 45min. The volume of the supernatant was recorded in a 10 ml graduated cylinder and used for determinations of water absorption; WAC was expressed as the weight of water bound by 1g dry flour.

$$WAC = (W_2 - W_1) \div W_s$$

Where, W_s is the weight of the sample, W_1 is the weight of centrifuge tube plus sample and W_2 is the weight of centrifuge tube plus the sediments.

3.3.6.4 Oil-Holding capacity absorption (OHCA)

Oil absorption capacity was determined according to the methods of Chau and Huang (2003). One g of each sample flours were measured and mixed with 10 ml of oil (pure soybean oil). The mixture was stirred for 30 min at room temperature. After sample was centrifuged at 2500 rpm for 30 min, the supernatant was transferred to a graduated cylinder of 10 ml, where the volume was measured. OHCA was expressed as the weight of oil bound by 1g dry flour.

$$OHCA = (W_2 - W_1) \div W_s$$

Where, W_s is the weight of the sample, W_1 is the weight of centrifuge tube plus sample and W_2 is the weight of centrifuge tube plus the sediments.

3.3.7 Sensory evaluation

The sensory evaluation was carried out for consumer acceptance to evaluate the sensory attributes of gruels prepared from fermented and unfermented sorghum, maize, sorghum/soybean blend and maize/soybean blend flours. Gruels were prepared by mixing 20g of each sample dissolved in 400ml tap water and boiled at 92^0C for 15min in the experimental kitchen of Ethiopian Health and Nutrition Research Institute (EHNRI). The boiled gruels were allowed to cool to about 45°C. All three digits coded samples presented in a random order assigned to each panelist in white and transparent glass cups at ambient temperature.

Ten semi-trained mother panelists were randomly selected from staff of the Food Science and Nutrition Research Directorate, Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa, Ethiopia. The panelists were given the fundamental information about the purpose and objective of the test, so that the selection of panelists was based on basic requirements of a panelist, such as availability for the entire period of evaluation, interest, willing to serve. The health status of panelists was also considered during panelist selection (not suffering from colds and allergies that affect their sensitivity for the product).

During the orientation, the panelists were familiarized with method, scorecard and the product being used in the study. The panel members were seated individually in isolated booth which provided a quiet and comfortable environment and asked to indicate their degree of liking the gruel samples based on color, aroma, taste, texture and over all acceptability using a nine point hedonic scale where 1 = dislike extremely and 9 = like extremely (Inyang and Idoko, 2006). The panelists were provided with odor the bottled water to rinse their mouth before and after evaluating each sample. Finally data were collected and analyzed statistically.

3.3 Structure of the thesis experiment

The overall framework of experiments of the thesis is shown in Figure. 3.3. It generally shows raw material collection and preparation, formulation, processing methods, sample analysis and sensory evaluation of the product.

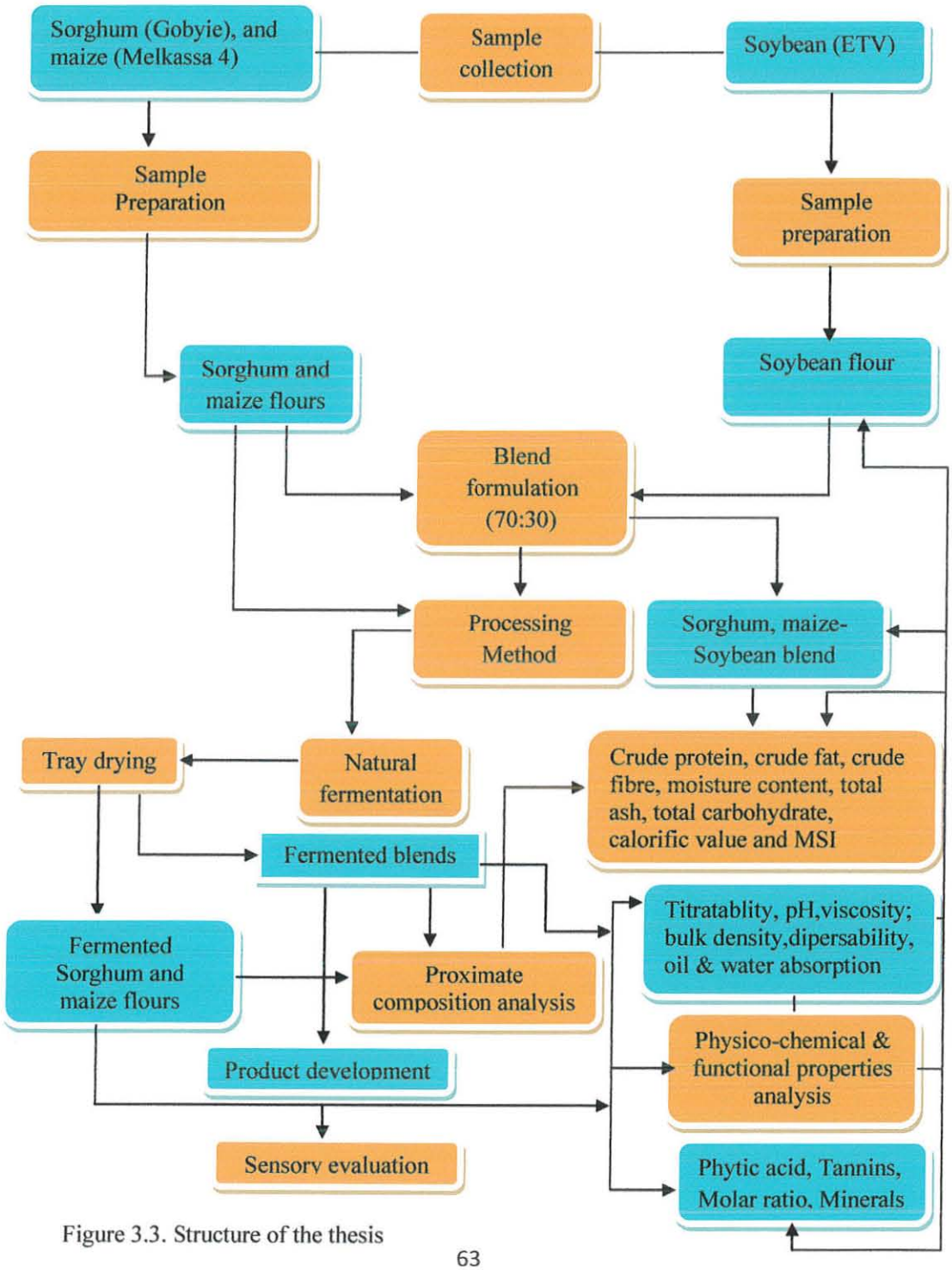


Figure 3.3. Structure of the thesis

3.3.9 Selection criteria for determining optimal complementary food

A ranking system using six nutritional criteria, i.e., protein content, energy value, calcium: phosphorous ratio, total essential amino acids, biological values and sensory attributes was devised to determine optimal blend combination according to the method modified by Griffith *et al.* (1998)

3.3.10 Statistical analysis

Each determination was carried out in duplicate and results were reported as an averaged value (mean ± standard deviation). Data was analyzed by T-Test and one-way analysis of variance (ANOVA) model using SPSS Version 20. Differences between treatments were determined by the Fisher's Least Significance Difference (LSD) was used for multiple mean comparison tests. Statistical significance was set at $p<0.05$.

CHAPTER FOUR

Results and Discussion

Proximate nutrients composition, antinutritional factors, sensory characteristics, physico-chemical and functional properties and effect of natural fermentation of diets were studied. The use of locally available foodstuff to formulate diet agrees with different guideline and criteria to prepare food for infants and children in order to ensure availability and affordability of foodstuff in various low income communities. Such guideline are particularly important in a developing country where gross malnutrition has largely been attributed to inadequate intake of food materials due to inability of parents or families to afford proper diets particularly animal source foods. In some cases, ignorance about essential food materials that could provide a balance diet has resulted in poor diet. Carbohydrate, fat, protein, energy and minerals elements are particularly important both in quantity and quality when formulating diets for infants, where requirements must be met for rapid growth, development and maintenance of good health.

4.1 Proximate Nutrient Composition

4.1.1 Moisture content

The moisture content of the Diet 1, Diet 2, Diet 3, Diet 4 and Diet 5, Diet 6, Diet 7, Diet 8 and Diet 9 are presented in Table 4.1 and 4.2 respectively. The moisture content of control (Diet1) and blended (Diet 3) food samples ranged between 4.6±0.00g/100g and 4.1±0.11g/100g respectively whereas the moisture content of fermented food samples ranged between 4.8±0.28g/100g and 4.6±0.00g/100g for Diet 2 and Diet 4 respectively. The moisture content of the blended food (Diet 3) (4.1±0.11g/100g) was lower compare with other fermented food samples, which are Diet 2 (4.8±0.28g/100g) and Diet 4 (4.6±0.00g/100g), and control food sample (Diet 1) (4.6±0.00g/100g). The reduction of moisture content in Diet 3 was due to the blended with soybean flour which is similarly with those observed by Aremu *et al.* (2011). Fermentation did not significantly ($p<0.05$) affect the moisture content of Diet 1 and increased moisture content of Diet 3 from 4.1±0.11g/100g to 4.6±0.00g/100g after 72hrs of fermentation time. This is may be due to climatic difference during the experiment.

Table 4.1: Proximate Nutrient Composition (g/100g Dry weight matter) of fermented sorghum and sorghum/soybean blend flours.

Nutrient/Sample code	Diet 1	Diet 2	Diet 3	Diet 4
Moisture	4.60 ± 0.00^{b}	4.80 ± 0.28^{b}	4.10 ± 0.11^{a}	4.6 ± 0.00^{b}
Total Ash	1.27 ± 0.28^{b}	0.75 ± 0.18^{a}	2.12 ± 0.23^{c}	1.28 ± 0.42^{b}
Crude protein	9.00 ± 0.49^{a}	11.83 ± 0.19^{b}	14.31 ± 0.46^{c}	16.73 ± 0.21^{d}
Crude fat	3.45 ± 0.01^{a}	6.53 ± 0.12^{b}	13.30 ± 0.86^{c}	9.78 ± 0.79^{d}
Crude fibre	2.39 ± 0.05^{b}	1.58 ± 0.02^{a}	3.88 ± 0.34^{c}	4.41 ± 0.63^{d}
Carbohydrate	83.95^{d}	79.28^{c}	66.39^{a}	67.80^{b}
Energy value (kcal)	402.52^{a}	422.96^{b}	442.46^{d}	426.02^{c}

Values are means of two duplicates ± SD. Means followed by the same letter in a row are not significantly different ($p<0.05$). Sample code as in Table 3.1

Table 4.2: Proximate Nutrient Composition (g/100g Dry weight matter) of fermented maize and maize/soybean blend flours.

Nutrient/Sample code	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Moisture	6.39 ± 0.01^{c}	7.06 ± 0.11^{d}	5.4 ± 0.00^{b}	5.6 ± 0.28^{b}	1.5 ± 0.14^{a}
Total Ash	0.8 ± 0.28^{a}	1.2 ± 0.00^{b}	2.6 ± 0.28^{c}	1.2 ± 0.01^{b}	4.41 ± 0.55^{d}
Crude protein	7.34 ± 0.00^{a}	10.61 ± 0.48^{b}	12.34 ± 0.34^{c}	14.57 ± 0.23^{d}	41.39 ± 3.07^{e}
Crude fat	4.35 ± 0.79^{a}	4.70 ± 0.35^{a}	11.50 ± 0.64^{c}	9.31 ± 0.60^{b}	30.57 ± 2.12^{d}
Crude fibre	1.95 ± 0.05^{a}	1.80 ± 0.10^{a}	4.11 ± 0.01^{b}	4.12 ± 0.13^{b}	9.54 ± 2.30^{c}
Carbohydrate	85.56^{e}	81.69^{d}	69.45^{b}	71.20^{c}	14.09^{a}
Energy value (kcal)	410.43^{a}	411.71^{b}	430.57^{d}	425.13^{c}	497.63^{e}

Values are means of two duplicates ± SD. Means followed by the same letter in a row are not significantly different ($p<0.05$). Sample code as in Table 3.1

As can be observed from Table 4.2, the moisture content of control (Diet 5) and blended food samples (Diet 7) ranged between 6.39±0.01g/100g and 5.4±0.00g/100g respectively whereas the moisture of fermented food samples ranged between 7.06±0.11g/100g and 5.6±0.28g/100g for Diet 6 and Diet 8 respectively. The moisture content of Diet 6 (7.06±0.11g/100g) was higher compared with control food sample (Diet 5) (6.39±0.01g/100g), fermented food sample, which is Diet 8 (5.6±0.28g/100g), and blended food, which is Diet 7 (5.4±0.00g/100g), and Diet 9 (1.5±0.14g/100g).

Fermentation did not significantly ($p<0.05$) affect the moisture content of Diet 7 and increased the moisture content of Diet 5 from 6.39±0.01g/100g to 7.06±0.11g/100g after 72hrs of fermentation time. The lower moisture content of all diets is a desirable phenomenon, as it will enhance keeping quality of diets since water for microbial activity is low. Low moisture content in diets increased the storage periods of food products while high moisture contents in food encourage microbial growth; hence, food spoilage (Temple *et al.*, 1996). Amankwah *et al.* (2009a) reported that removal of moisture generally increases concentrations of nutrients and can make some nutrients more available.

Amankwah *et al.* (2009b) reported that moisture content is used as a quality factors for prepared cereals which should have 3-8g/100g moisture content, therefore the maximum moisture content attained in Diet 6 is 7.06g/100g. The relative increase in the moisture content in Diet 4 and 6 may be the addition of water during fermentation process and attributed a variation in the treatment during the drying process of the diet. However, these values are in agreement with the values reported by Amankwah *et al.* (2009b). Such low moisture content of foods prevents microbial activity and extends the shelf life of the diet (Kikafunda *et al.*, 2006). According to WHO specification, the maximum requirements of moisture contents of maize-soy blends is 10%, therefore the value indicated in Table 4.1 and 4.2 is agreement with this value. It is therefore recommended that small quantities of the diets be prepared at a time especially during humid seasons.

4.1.2 Total ash content

The total ash content of Diet 1 and 3 ranged between 1.27±0.28g/100g and 2.12±0.23g/100g respectively whereas the total ash content of Diet 2 and Diet 4 ranged between 0.75±0.18g/100g and 1.28±0.42g/100g respectively (Table 4.1). The ash content of Diet 3 (2.12±0.23g/100g) was significantly ($p<0.05$) higher than that of Diet 1 (1.27±0.28g/100g) due to supplementation by soybean flour sample and also higher than Diet 2 (0.75±0.18g/100g). Fermentation significantly ($p<0.05$) decreased the total ash content of Diet 1 and Diet 3 from ranged 1.27±0.28g/100g to

0.75±0.18g/100g and from ranged 2.12±0.23g/100g to 1.28±0.42g/100g respectively after 72hrs of fermentation time (Table 4.1). These results are in agreement with Mihret (2009) observed that the ash content in a sorghum was significantly decreased after fermentation.

The total ash content of Diet 5 and 7 ranged between 0.8±0.28g/100g and 2.6±0.28g/100g respectively whereas the total ash content of Diet 6, Diet 8 and Diet 9 ranged between 1.2±0.00g/100g,1.2±0.01g/100g and 4.41±0.55g/100g respectively (Table 4.2). Ash content of Diet 7 (2.6±0.28g/100g) was significantly ($p<0.05$) higher than Diet 5 (0.8±0.28g/100g) due to the effect of supplementation with soybean flour. Fermentation significantly ($P<0.05$) increased the ash content of Diet 5 from ranged 0.8±0.28g/100g to 1.2±0.00g/100g whereas ash content of Diet 7 decreased from ranged 2.6±0.28g/100g to 1.2±0.01g/100g after 72hrs of fermentation time.

Fermentation significantly ($P<0.05$) increased the ash content of Diet 5 (maize) after 72hrs of fermentation time which are similar to the values reported on the effect of fermentation and malting on viscosity of maize-soybean weaning blends (Amankwah *et al.*, 2009a). Fermentation significantly ($P<0.05$) decreased on the ash content of both blend diets which are similar to the values reported on the production of legumes-fortified weaning food (Egounlety, 2002) and development and Nutritional Assessment of a Weaning Food from Sorghum and Oil-Seeds (Lalude and Fashakin, 2006). In this experimental study all values are acceptable by the Protein Advisory Group which recommended that the ash content of weaning food should not exceed 5g/100g (Munasinghe *et al.*, 2013). The ash content decreases in diets might be due to vegetative loss during fermentation. This could also be that it leached into the fermentation or microflora used it for metabolism (Beebe *et al.*, 2000).

4.1.3 Crude protein content

The crude protein content of Diet 1 and Diet 3 ranged between 9.00±0.49g/100g and 14.31±0.46g/100g respectively whereas the crude protein content of Diet 2 and Diet 4 ranged between 11.83±0.19g/100g and 16.73±0.21g/100g respectively. The crude protein content of Diet 3 (14.31±0.46g/100g) was significantly ($p<0.05$) higher than Diet 1 (9.00±0.49g/100g) due to supplementation with soybean flour which is a good source of protein recommended by Omueti *et al.*(2009a) in the formulation of infants food and also higher than Diet 2 with value 11.83±0.19g/100g (Table 4.1).

Fermentation significantly ($p<0.05$) increased crude protein content of Diet 1 and Diet 3 from 9.00±0.49g/100g to 11.83±0.19g/100g and from 14.31±0.46g/100g to 16.73±0.21g/100g respectively after 72hrs of fermentation time. This is agreement with value (15.9g/100g) reported by Akanbi *et al.*) on Quality assessment of selected cereal/soybean mixtures in "ogi" production. Chavan *et al.* (1988) reported that the content and quality of protein in sorghum cultivars may be improved by fermentation. Protein is particularly important both in quantity and quality for rapid growth and development of a child. Infant's amino acid requirements are proportionately higher than those of adults. In addition histidine is essential for infant at a level surpassed in both breastfeeding and bottle-feeding. The protein content increases during the fermentation may be due to the fact that, the proteolytic activities of enzymes produced by microorganisms during fermentation increases the bioavailability of amino acids.

The crude protein content of Diet 5 and Diet 7 ranged between 7.34±0.00g/100g and 12.34±0.34g/100g respectively whereas the crude protein content of Diet 6 and Diet 9 ranged between 10.61±0.48g/100g and 41.39±3.07g/100g respectively which is similar to the value reported by Snyder and Kwon (1987) that the content of soy protein in soybeans ranged between 38-44% is much larger than that of cereals ranged between 8-15% and, the protein content of Diet 8 ranged between 14.57±0.23g/100g (Table 4.2).

The crude protein value of Diet 7 (12.34±0.34g/100g) was significantly ($p<0.05$) higher than Diet 5 (7.34±0.00g/100g) due to the effect of blending with soybean flour and also higher than Diet 6 (10.61±0.48g/100g). Fermentation also significantly ($p<0.05$) increased the protein content of Diet 5 and 7 from 7.34±0.00g/100g to 10.61±0.48g/100g and from 12.34±0.34g/100g to 14.17±0.23g/100g respectively after 72hrs of fermentation time. The protein increase might be also due to the fact that some amino acids are produced in excess of the requirement during protein synthesis and these tend to accumulate in free amino acid pool (Marero *et al.*, 1988). Other researchers have attributed the increase to the degradation of storage protein and synthesis of new protein and other materials (King and Puwastien, 1987). The poor protein levels of traditional complementary foods have been a major concern in infant feeding (Brown *et al.*, 1998). Use of the formulation could serve as a practical means of upgrading the protein levels of the traditional sorghum and maize based complementary foods.

4.1.4 Crude fat content

The crude fat content of Diet 1 and Diet 3 ranged between 3.45±0.01g/100g and 13.30±0.86g/100g respectively whereas the crude fat content of Diet 2 and Diet 4 ranged between 6.53±0.12g/100g and 9.78±0.79g/100g respectively (Table 4.1). The crude fat content of Diet 3 (13.30±0.86g/100g) was significantly ($p<0.05$) higher than that of Diet 1 (3.45±0.01g/100g). Fermentation significantly ($p<0.05$) decreased crude fat content of Diet 3 from 13.30±0.86g/100g to 9.78±0.79g/100g which agreement with the value reported on Quality Assessment of Selected Cereal-Soybean Mixtures in "ogi" production (Akanbi *et al.*) and increased crude fat content value of Diet 1 from 3.45±0.01g/100g to 6.53±0.12g/100g after 72hrs of fermentation time which agreement with previously research finding (Dicko *et al.*, 2006) that the fat content of sorghum flour range from 1.5% to 6%, the crude fat content of sorghum flour was reported to be 3.25% (Gobezie *et al.*, 1997) and 3.2% (Leder, 2004).

The crude fat contents of Diet 5 and Diet 7 ranged between 4.35±0.79g/100g and 11.50±0.64g/100g respectively whereas the crude fat content of Diet 6, Diet 8 and Diet 9 ranged between 4.70±0.35g/100g, 9.31±0.60g/100g and 30.57±2.12g/100g respectively (Table 4.2). The crude fat content of Diet 7 (11.50±0.64g/100g) was significantly ($p<0.05$) higher than that of Diet 5 (4.35±0.79g/100g). Fermentation significantly ($p<0.05$) decreased the crude fat content of Diet 7 from 11.50±0.64g/100g to 9.31±0.6g/100g and did not affect the crude fat content of Diet 5 after 72hrs of fermentation time. However, these values are in agreement with the values reported on formulation of weaning food from fermented maize, rice, soybean and fishmeal (Amankwah *et al.*, 2009b). The reduction of fat content of Diet 3 and Diet 7 after fermentation may be attributed to the activities of micro-organisms on these nutrients in utilizing them to synthesize protein for their growth (Sade, 2009).

Fat composition of Diet 4 with (9.78±0.79g/100g) and Diet 8 with (9.31±0.6g/100g) correspond to recommend by Protein Advisory group that fat level for complementary foods should not more than 10% (Munasinghe *et al.*, 2013). High crude fat content observed in Diet 3 with 13.30±0.86g/100g and Diet 7 with 11.50±0.64g/100g. This could be due to the inclusion of some oil-dense legumes such as soybean flour. Fats contribute substantially to energy value of food as well as provide essential fatty acid for optimal neurological, immunological and functional developments in children (Guthrie, 1989). Fats are a major energy source for the infant body. Fatty acids are also involved in many other vital processes in the body (e.g. structural components of cell membranes, precursors for bioactive molecules, regulators of enzyme activities, regulation of gene expression).

Furthermore, high fat content of a food sample can affect its shelf stability. This is because fat can undergo oxidative deterioration, which leads to per-oxidation of polyunsaturated fatty acid (rancidity) and food spoilage that would impact unpleasant odour and reduced intake of food and nutrient. Hence a food sample with high fat content is more liable to spoilage than one with a lower fat content. This is particularly important in countries where household electricity and facilities for refrigeration are luxury for most people. On the other hand, the high fat content in the formulated foods may lead to excessive intake of calorie, more so that carbohydrate content of the diet are sufficiently high. This may therefore lead to a rapid gain in weight, which is as undesirable in infants as in adults. Furthermore, high intake of fat especially saturated fatty acids has been shown to increase the level of cholesterol in the blood (Amankwah *et al.*, 2009b).

4.1.5 Crude fibre content

Complementary foods with low fibre content are very important since it helps in the safety of children considering their stomach capacity since they have to consume more to get satisfied to meet their daily energy requirement (Eka and Edijala, 1972). The fibre content of Diet 1 and Diet 3 ranged between 2.39±0.05g/100g and 3.88±0.34g/100g respectively whereas the crude fibre content of Diet 2 and Diet 4 ranged 1.58±0.02g/100g and 4.41±0.63g/100g respectively (Table 4.1). The crude fibre content of Diet 3 (3.88±0.34g/100g) was significantly ($p<0.05$) higher than Diet 1 (2.39±0.05g/100g). This is attributed to the high fibre content of soybean flour compared to Diet 1.

Fermentation significantly ($p<0.05$) decreased the crude fibre content of Diet 1 from 2.39±0.05g/100g to 1.58±0.02g/100g and increased the crude fibre content of Diet 3 from 3.88±0.34g/100g to 4.41±0.63g/100g after 72hrs of fermentation time (Table 4.1) and, which are in agreement with the value reported on Quality Assessment of Selected Cereal-Soybean Mixtures in "ogi" production (Akanbi *et al.*). The loss of fibre in Diet 1 was due to hydrolysis and leaching into fermentation medium or the microflora used it for metabolism (Odunfa, 1987). Mihiret (2009) observed that sorghum crude fibre decreased after fermentation. The reduction of crude fibre content might also be due to enzymatic degradation of the fibrous material during fermentation (Ikenebomeh *et al.*, 1986).

The crude fibre contents of Diet 5 and Diet 7 ranged between 1.95±0.05g/100g and 4.11±0.01g/100g respectively whereas the fibre content of Diet 6, Diet 8 and Diet 9 ranged between 1.8±0.10g/100g, 4.12±0.13g/100g and 9.54±2.30g/100g respectively (Table 4.2). The crude fibre content of Diet 7 (4.11±0.01g/100g) was significantly ($p<0.05$) higher than that of Diet 5 (1.95±0.05g/100g). This is attributed to the high fibre content of soybean flour compared to Diet 5.

Fermentation did not significantly ($p<0.05$) affect crude fibre content of both Diet 5 and Diet 7 after 72hrs of fermentation time (Table 4.2). However, these values are similar to the values reported for complementary foods contain about 5% crude fibre as suggested by Ijarotimi and Keshinro (2012). The low crude fibre is nutritionally appreciated because it traps less protein and carbohydrates (Balogun and Fetuga, 1986). The crude fibre content of infant foods is expected to be low (Olorunfemi *et al.*, 2006) as food with high fibre content tends to cause indigestion in babies. Hence, samples with low fibre content were rated good as potential complementary foods. Fermentation as a process is promising to meet crude fibre standards in the preparation of complementary foods from locally available cereals.

On the other hand, high fibre content or excessive intake may reduce the availability of nutrients such as calcium and zinc especially in food that marginal in these elements. High fibre content is also known to increase viscosity of food. This is particularly worth considering in infant feeding because highly viscous foods would reduce intake (Rolland-Cachera *et al.*, 1999).

4.1.6 Total carbohydrates content

Carbohydrate content of Diet 1 and Diet 3 ranged 83.95g/100g and 66.39g/100g respectively whereas the carbohydrate content of Diet 2 and Diet 4 ranged 79.28g/100g and 67.80g/100g respectively (Table 4.1). The carbohydrate content of Diet 1 (83.95) was significantly ($p<0.05$) higher than that of Diet 3 (66.39). This is due to high content of carbohydrates in Diet 1. As the blend ratio increases the amount of carbohydrate decreases. The total carbohydrate contents of Diet 5 and 7 ranged 85.56g/100g and 69.45g/100g respectively whereas the total carbohydrate content of Diet 6, Diet 8 and Diet 9 ranged 81.69g/100g, 71.2g/100g and 14.09g/100g respectively (Table 4.2). The total carbohydrate content of Diet 7 (69.45) was significantly ($p<0.05$) lower than that of Diet 5 (85.56). This is due to the high content of carbohydrates in Diet 5.

Fermentation significantly ($p<0.05$) decreased the total carbohydrate content of Diet 1 from 83.95g/100g to 79.28g/100g in agreement with the values reported from the effect of natural fermentation on some anti-nutritional factors, minerals, proximate composition and sensory characteristics in sorghum based weaning food (Mihiret, 2009) and decreased the carbohydrate content of Diet 5 from 85.56g/100g to 81.69g/100g which are comparable with the value reported from effect of fermentation on quality protein maize-soybean blends for the production of weaning food (Bekele, 2011) after 72hrs fermentation time (Table 4.1 and 4.2 respectively).

Fermentation significantly ($p<0.05$) increased the total carbohydrate content of Diet 3 from 66.39g/100g to 67.80g/100g and increased the total carbohydrate content of Diet 7 from 69.45g/100g to 71.2g/100g. These values are in agreement with the value reported on Quality Assessment of selected cereal-soybean mixtures in "ogi" production (Akanbi *et al.*) and comparable with the values (60.85% and 61.99%) reported by Amankwah *et al.* (2009b) for different blend ratio in the formulation of weaning food from fermented maize, rice, soybean and fishmeal.

The decrease in carbohydrates calculated by difference could be due to the fact that starch and soluble sugars are principal substances for fermenting microorganisms; therefore degradation and a subsequent decrease in starch content are expected to occur (Ejigui *et al.*, 2005). In general, fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly and oligosaccharides (Katongole, 2008). The non-digestible carbohydrates (NDC) which are fibres decrease as the total carbohydrates decreases.

The cereal-legume based diets were found contain carbohydrate in the range of 66.39-71.2g/100g. According to Gibney *et al.* (1995), when formulating diets for infants, the ratio of carbohydrate to fat and protein must be taken into consideration. This is because a high protein and low carbohydrate diet would mean that the amino acids of the protein will be diverted to glucose synthesis and the body is deprived of protein meant for body building with resultant wasting and stunting. Values obtained in this study seem to recommend that carbohydrate will be sufficient to meet requirements.

4.1.7 Energy value

The calories in an infant's diet are provided by protein, fat and carbohydrates (Amankwah *et al.*, 2009). The energy value of Diet 1 and Diet 3 ranged 402.52kcal and 442.46kcal respectively whereas the energy of Diet 2 and Diet 4 ranged 422.96kcal and 426.02kcal respectively (Table 4.1). The energy value of Diet 3 (442.46kcal) was significantly ($p<0.05$) higher than that of Diet 1 (402.52kcal). This is due to high fat and protein content of Diet 3.

Fermentation significantly ($p<0.05$) increased the energy value of Diet 1 from 402.52kcal to 422.96kcal in agreement with the values reported from the effect of natural fermentation on some anti-nutritional factors, minerals, proximate composition and sensory characteristics in sorghum based weaning food (Mihiret, 2009) and decreased the amount of energy value of the Diet 3 from 442.46kcal to 426.02kcal in agreement with the values reported from Quality Assessment of Selected Cereal-Soybean Mixtures in "ogi" production (Akanbi *et al.*) after 72hrs of fermentation time in Table 4.1. The energy value of Diet 5 and Diet 7 ranged 410.43kcal and 430.57kcal respectively whereas the energy value of Diet 6, Diet 8 and Diet 9 ranged 411.71kcal, 425.13kcal and 497.63kcal respectively (Table 4.2). The total energy value of Diet 7 (430.57kcal) was significantly ($p<0.05$) higher than that of Diet 5 (410.43kcal). This is due to the high fat and protein content of Diet 7.

Fermentation significantly ($p<0.05$) increased the energy value of Diet 5 from 410.43kcal to 411.71kcal which is in agreement with the values reported from effect of fermentation on quality protein maize-soybean blends for the production of weaning food (Bekele, 2011) and decreased the energy value of Diet 7 from 430.57kcal to 425.13kcal as shown in the Table 4.2. However, the values are agreement with the values reported from effect of fermentation on quality protein maize-soybean blends flour for the production of weaning food (Bekele, 2011). The value is also in agreement with WHO specification minimum requirement of (380kcal) for the weaning food from corn-soya blend (CSB) and as reported by Onilude *et al.* (1999) they are comparable with

the values of unfermented blend (418.0kcal) and lower than the fermented ones (464.2kcal) for the composite blend of cereal and soybean for infant weaning food.

Low calories as a result of inadequate consumption of energy giving nutrients especially carbohydrates and fats will deprive the body of needed basal metabolic processes and strength for physical activities. It is believed that frequent consumption of the diets along with breast-milk, would satisfy the daily energy requirements of infants. Nutritionally, protein contents and energy values of experimental food samples fulfill the specification guidelines for the young child complementary food formulations. In comparison, the nutrient-dense of formulated diets were higher than that of the traditional complementary foods that characterize with low energy and nutrient density (King and Ashworth, 1987). Hence, it could be deduced that the formulated diets were better than ogi, which has been implicated in the eatiology of malnutrition among children who were solely weaned on ogi (Okoye, 1992).

Epidemiological studies have investigated that malnutrition constitutes a serious nutritional and health problem for children between 6 to 24 months of age, a period of complementary feeding, in developing countries, due to poor complementary feeding practices (Daelmans and Saadeh, 2003). This nutrition problem is responsible for growth retardation, increase in morbidity and mortality rate among children falling within the low income families who cannot afford the high cost of fortified nutritious proprietary complementary foods (Ijarotimi and Keshinro, 2012).

4.2 Mineral Composition of fermented sorghum, maize, sorghum/soybean blend and maize/soybean blend flours

Calcium is an important mineral for bone and teeth formation (Smith, 2005) as well as body structure and in blood clotting. Its deficiency can lead to rickets in infant and children. It plays a corrective role when K, Mg or Na is in excessive amount in the body (Fleck, 1976). The calcium value of Diet 1 and Diet 3 ranged between 18.10±1.60mg/100g and 231.67±2.74mg/100g respectively whereas the calcium value of Diet 2 and Diet 4 ranged between 18.61±1.01mg/100g and 186.93±2.47mg/100g respectively (Table 4.3). The calcium of Diet 3 (231.67±2.74mg/100g) was significantly ($p<0.05$) higher than Diet 1 (18.10±1.60mg/100g) due to supplementation of soybean flour in diet 3.

Table 4.3: Mineral composition of fermented sorghum and sorghum/soybean blend flours (mg/100g).

Sample code	Ca	Fe	Zn
Diet 1	18.10±1.60^{a}	4.05±0.14^{a}	1.74±0.03^{a}
Diet 2	18.61±1.01^{a}	4.31±1.62^{a}	1.78±0.18^{a}
Diet 3	231.67±2.74^{c}	5.15±0.52^{b}	3.67±0.06^{c}
Diet 4	186.93±2.47^{b}	4.27±0.04^{a}	2.60±0.06^{b}

Values are means of two duplicates ± SD. Means followed by the same letter in a Column are not significantly different ($p<0.05$). Sample code as in Table 3.1

Table 4.4: Mineral composition of fermented maize and maize/soybean blend flours (mg/100g).

Sample code	Ca	Fe	Zn
Diet 5	9.83±0.38^{b}	2.57±0.59^{a}	1.78±0.41^{b}
Diet 6	5.64±0.06^{a}	2.50±0.11^{a}	1.21±0.03^{a}
Diet 7	217.25±3.42^{d}	4.30±0.40^{b}	3.44±0.04^{c}
Diet 8	115.75±0.23^{c}	2.52±0.29^{a}	1.62±0.13^{b}
Diet 9	474.79±5.25^{e}	5.72±0.90^{c}	5.91±0.95^{d}

Values are mean of two duplicates ± SD. Means followed by the same letter in a column are not significantly different ($p<0.05$). Sample code as in Table 3.1

Fermentation did not significantly ($p<0.05$) affect calcium content of Diet 1 and decreased the calcium content of Diet 3 from 231.67±2.74mg/100g to 186.93±2.47mg/100g after 72hrs fermentation time which is in agreement with value reported on evaluation of nutritional and microbiological status of co-fermented cereals/cowpea 'OGI'(Oyarekua, 2011). The calcium content of Diet 5 and Diet 7 ranged between 9.83±0.38mg/100g and 217.25±3.42mg/100g respectively whereas the calcium content of Diet 6, Diet 8 and Diet 9 ranged between 5.64±0.06mg/100g,115.75±0.23mg/100g and 474.79±5.25mg/100g respectively (Table 4.4). The

calcium content of Diet 7 (217.25±3.42mg/100g) was significantly ($p<0.05$) higher than that of Diet 5 (9.83±0.38mg/100g) due to supplementation of soybean flour in diet 7.

Fermentation was significantly ($p<0.05$) decreased the calcium content of Diet 5 from 9.83±0.38mg/100g to 5.64±0.06mg/100g after 72hrs of fermentation time which is in agreement with the value reported on effect of co-fermentation on nutritive quality and pasting properties of maize/cowpea/sweet potato as complementary food (Oyarekua, 2013). Fermentation also significantly ($p<0.05$) decreased the calcium content of Diet 7 from 217.25±3.42mg/100g to 115.75±0.23mg/100g after 72hrs of fermentation time. Calcium concentration was high in Diet 3 and Diet 7 with value 231.67±2.74mg/100g and 217.25±3.42mg/100g respectively. However; these values are not enough to meet RDA from complementary foods for children.

Globally, the highest prevalence of iron deficiency is found in infants and children. The weaning period in infants is especially critical because of very high iron requirements in relation to energy requirements. Thanks to better information and access to fortified cereals for infants and children, the iron situation has markedly improved in these groups in most industrialized countries where the highest prevalence of iron deficiency are observed in infants. In developing countries, however, the iron situation is very critical in many groups, especially in the weaning period. Iron nutrition is a great importance for the adequate development of the brain and other tissues such as muscles, which are finally differentiated early in life (Hallberg *et al.*, 1993).

Iron content of Diet 1 and Diet 3 ranged between 4.05±0.14mg/100g and 5.15±0.52mg/100g respectively whereas the iron content of Diet 2 and Diet 4 ranged between 4.31±1.62mg/100g and 4.27±0.04mg/100g respectively. Fermentation significantly ($p<0.05$) decreased the iron content of Diet 3 from 5.15±0.52mg/100g to 4.27±0.04mg/100g and did not affect iron content of Diet 1 after 72hrs of fermentation time (Table 4.3) which is in agreement with the value reported from comparative studies of co-fermented maize/pigeon pea and maize/mucuna infants complementary foods (Adenike).

Iron content of Diet 5 and Diet 7 ranged between 2.57±0.59mg/100g and 4.30±0.40mg/100g respectively whereas iron content of Diet 6, Diet 8 and Diet 9 ranged between 2.50±0.11mg/100g, 2.52±0.29mg/100g and 5.72±0.90mg/100g respectively (Table 4.4). The iron content of Diet 7 (4.30±0.40mg/100g) was significantly ($p<0.05$) higher than that of Diet 5 (2.57±0.59mg/100g). Fermentation was significantly ($p<0.05$) decreased iron content of Diet 7 from 4.30±0.40mg/100g to 2.52±0.29mg/100g and did not affect iron content of Diet 5 after 72hrs of fermentation time which is lower than the value reported on effect of co-fermentation on nutritive quality and pasting properties of maize/cowpea/sweet potato as complementary food (Oyarekua, 2013).

Consumption of diet 1, 2, 3, 4 and 7 may be lead to significant increase in the growth of 12-24 months infants because these values are in agreement with the value recommended iron intake is 0.8mg/100kcal from 12 to 24 months (Monte and Giugliani, 2004).

Zinc is present in all tissues of the body and is a component of more than 50 enzymes (Bender, 1992). Zinc is necessary for protein and blood formation and to maintain vitamin A concentration in plasma. It is a limiting factor in the growth of severely malnourished infants especially in developing countries; because the diets are low in animal products and high in anti nutrients. Deficiency of zinc may negatively affect the behavioral development and growth of infants. Zinc loss might also occur as a result of diarrhea (WHO/NUT/98). Zinc content of Diet 1 and Diet 3 ranged between 1.74±0.03mg/100g and 3.67±0.06mg/100g respectively whereas the zinc content of Diet 2 and Diet 4 ranged between 1.78±0.18mg/100g and 2.60±0.06mg/100g respectively (Table 4.3). Zinc content of Diet 3 (3.67±0.06mg/100g) was significantly ($p<0.05$) higher than that of Diet 1 (1.74±0.03mg/100g).

Fermentation did not significantly ($p<0.05$) affect zinc content of Diet 1 and decreased the zinc content of Diet 3 from 3.67±0.06mg/100g to 2.60±0.06mg/100g after 72hrs of fermentation time which is agreement with the value reported on evaluation of nutritional and microbiological status of co-fermented cereals/cowpea 'OGI'(Oyarekua, 2011). Zinc content of Diet 5 and Diet 7 ranged between 1.78±0.41mg/100g and 3.44±0.04mg/100g respectively whereas zinc value of Diet 6, Diet 8 and Diet 9 ranged between 1.21±0.03mg/100g, 1.62±0.13mg/100g and 5.91±0.95mg/100g respectively (Table 4.4). Zinc content of Diet 7 (3.44±0.04mg/100g) was significantly ($p<0.05$) higher than that of Diet 5 (1.78±0.41mg/100g). Fermentation was significantly ($p<0.05$) decreased the zinc content of Diet 5 and Diet 7 from 1.78±0.41mg/100g to 1.21±0.03mg/100g and from 3.44±0.04mg/100g to 1.62±0.13mg/100g respectively after 72hrs of fermentation time which is agreement with the value reported on effect of co-fermentation on nutritive quality and pasting properties of maize/cowpea/sweet potato as complementary food (Oyarekua, 2013).

Consumption of Diet 3, Diet 4 and Diet 7 may be lead to significant increase in the growth of 11-24 months infants because these values are in agreement with (2.08mg/100g) standards required from complementary foods for 11-24 months based on daily consumption of 750ml/d (WHO/NUT/98) and lower than 10mg/100g required from supplementary foods for older infants and young children (Oyarekua, 2011).

Reduction of calcium, iron and zinc contents in diets may be ascribed due to microorganisms could have utilized some of hydrolyzed elements for their metabolic activities and lost through decantation and the minerals could have been lost in the fermentation medium and decant of fermentation water during the drying process (Retta, 2010).

4.2.1 Mineral safety index of Ca, Fe and Zn for fermented sorghum, maize, sorghum/soybean blend and maize/soybean blend flours (mg/100g).

The mineral safety index (MSI) values of the samples are shown in Table 4.5. The standards MSI for the minerals for infants are Ca (10), Fe (6.7) and Zn (33)(Oyarekua, 2011). For calcium, the mineral safety index values ranged from 0.15 (Diet 6)-11.87 (Diet 9) (soybean flour) which was normal in all Diets except Diet 9; only Diet 9 had negative difference (-1.87) between the standard and calculated MSI values. This indicates that consumption of Diet 9 alone by infant and children might lead to overload of calcium. High calcium intakes can lead to constipation, an increased chance for developing calcium kidney stones and may inhibit the absorption of iron and zinc from food (Houtkooper and Farrell, 2011). Zinc and iron values in all diets were comparable to the mineral safety index values for infants.

Table 4.5: Mineral safety index of Ca, Fe and Zn for fermented sorghum, maize, sorghum/soybean blend, maize/soybean blend flours (mg/100g).

	Mineral Safety Index								
Sample code	Ca			Fe			Zn		
	TV	CV	D	TV	CV	D	TV	CV	D
Diet 1	10	0.45	9.55	6.7	2.72	3.98	33	9.57	23.43
Diet 2	10	0.46	9.54	6.7	2.89	3.81	33	9.79	23.21
Diet 3	10	5.79	4.21	6.7	3.45	3.25	33	20.18	12.82
Diet 4	10	4.67	5.33	6.7	2.86	3.84	33	14.30	18.70
Diet 5	10	0.25	9.75	6.7	1.72	4.98	33	9.79	23.21
Diet 6	10	0.15	9.85	6.7	1.68	5.02	33	6.65	26.35
Diet 7	10	5.44	4.56	6.7	2.88	3.82	33	18.93	14.07
Diet 8	10	2.89	7.11	6.7	1.69	5.01	33	8.92	24.08
Diet 9	10	11.87	-1.87	6.7	3.84	2.86	33	32.50	0.500

TV = table value; CV = calculated value; D = difference.

4.3 Antinutritional factors

4.3.1 Tannin content

Tannin content of Diet 1 and 3 ranged between 19.36±2.14mg/100g and 26.16±1.07mg/100g respectively whereas the tannin content of Diet 2 and 4 ranged between 16.32±0.00mg/100g and 22.37±2.13mg/100g respectively (Table 4.6). The tannin content of Diet 3 (26.16±1.07mg/100g) was significantly ($p<0.05$) higher than Diet 1 (19.36±2.14mg/100g). This enlargement of tannin content is in accordance with the observation made by Ochieng'Anyango (2009) who reported that as the cowpea had higher tannin content than the sorghum, compositing increased the tannin content. Fermentation was significantly ($p<0.05$) decreased the tannin content of Diet 3 from 26.16±1.07mg/100g to 22.37±2.13mg/100g and fermentation also decreased the tannin content of Diet 1 from 19.36±2.14mg/100g to 16.32±0.00mg/100g after 72hrs of fermentation time which are in agreement with value reported on the effect of natural fermentation on some antinutritional factors, minerals, proximate composition and sensory characteristics in sorghum based weaning food (Mihiret, 2009).

Table 4.6: Antinutritional Factors (ANF) in fermented sorghum and sorghum/soybean blend flours.

Sample code	Antinutrient (mg/100g)	
	Tannin	Phytate
Diet 1	19.36 ± 2.14^{b}	362.43 ± 5.31^{c}
Diet 2	16.32 ± 0.00^{a}	194.06 ± 5.18^{a}
Diet 3	26.16 ± 1.07^{d}	275.27 ± 2.22^{b}
Diet 4	22.37 ± 2.13^{c}	193.44 ± 1.66^{a}

Values are means of two duplicates ± SD. Means followed by the same letter in a column are not significantly different ($p<0.05$). Sample code as in Table 3.1

Table 4.7: Antinutritional Factors (ANF) in fermented maize and maize/soybean blend flours.

Sample code	Antinutrient (mg/100g)	
	Tannin	Phytate
Diet 5	4.99 ± 3.21^{b}	254.67 ± 7.22^{d}
Diet 6	1.96 ± 1.06^{a}	253.65 ± 5.42^{d}
Diet 7	31.45 ± 4.27^{d}	219.75 ± 0.94^{c}
Diet 8	14.81 ± 2.13^{c}	173.40 ± 2.19^{b}
Diet 9	227.61 ± 3.21^{e}	104.09 ± 6.52^{a}

Values are means of two duplicates ± SD. Means followed by the same letter in a column are not significantly different ($p<0.05$). Sample code as in Table 3.1

Reduction in tannin contents was due to fermentation effect that have been caused by the activity of polyphenoloxidase or tanniase of fermenting microflora on tannins (Fagbemi *et al.*, 2005). Elkhier and Abd-Alraheem (2011) stated that the reduction in tannin contents of sorghum cultivars might be attributed to the activities of microorganisms during fermentation. Onilude *et al.*(2004) also observed reduction in both polyphenol and tannin content of cereal-soybean blends as a result of fermentation. The tannin of Diet 5 and Diet 7 ranged between 4.99±3.21mg/100g and 31.45±4.27mg/100g respectively whereas tannin content of Diet 6, Diet 8 and Diet 9 ranged between 1.96±1.06mg/100g, 14.81±2.13mg/100g and 227.61±3.21mg/100g respectively (Table 4.7). The tannin content of Diet 7 with value of 31.45±4.27mg/100g was significantly ($p<0.05$) higher than that of Diet 5 with value 4.99±3.21mg/100g.

Fermentation significantly ($p<0.05$) decreased the tannin of Diet 7 from 31.45±4.27mg/100g to 14.81±2.13mg/100g and also decreased the tannin content of Diet 5 from 4.99±3.21mg/100g to 1.96±1.06mg/100g after 72hrs of fermentation time (Table 4.7). This reduction is in accordance with the observation made by Charan and Kadam (1989) who reported that fermentation reduces tannin content of cereals. Lactic acid fermentation was also found to decrease tannin content in maize (Lopez *et al.*, 1983). The reduction in tannin content may be as a result of enzymatic activity of organisms whose hydrolyzing ability is enhanced by fermentation.

4.3.2 Phytate content

The phytate of Diet 1 and 3 ranged between 362.43±5.31mg/100g and 275.27±2.22mg/100g respectively whereas phytate content of Diet 2 and Diet 4 ranged between 194.06±5.18mg/100g and 193.44±1.66mg/100g respectively. Phytate content of Diet 3 (275.27±2.227mg/100g) was significantly ($p<0.05$) lower than that of Diet 1 with value of 362.43±5.317mg/100g (Table 4.6).

As indicated Table 4.6, fermentation significantly ($p<0.05$) decreased the phytate content of Diet 3 from 275.27±2.22mg/100g to 193.44±1.66mg/100g after 72hrs of fermentation time. Phytate content of Diet 1 also decreased from 362.43±5.31mg/100g to 194.06±5.18mg/100g after 72hrs of fermentation time. The results of this study are in agreement with those reported by Wedad *et al.* (2008) and Makokha *et al.* (2002), who stated that the fermentation of sorghum flour produces significant loss in phytate content. This result is also similar to that of Antony and Chandra (1997) who observed that fermentation of sorghum flour with starter from previously fermented sorghum flour achieved a desirable goal of reduced phytate and tannin content when compared to uncontrolled fermentation.

The phytate content of Diet 5 and Diet 7 ranged between 254.67±7.22mg/100g and 219.75±0.94mg/100g respectively whereas the phytate content of Diet 6, Diet 8 and Diet 9 ranged between 253.65±5.42mg/100g, 173.40±2.19mg/100g and 104.09±6.52mg/100g respectively (Table 4.7). The phytate content of Diet 7 (219.75±0.94mg/100g) was significantly ($p<0.05$) lower than that of Diet 5 with value of 254.67±7.22mg/100g. Fermentation significantly ($p<0.05$) decreased the phytate content of Diet 7 from 219.75±0.94mg/100g to 173.40±2.19mg/100g and did not affect the phytate content of Diet 5 after 72hrs of fermentation time as indicated in Table 4.7. The reduction of phytic acid content in diets may be due to hydrolysis of phytate by the activity of enzyme phytase into lower inositol phosphates which are believed to be activated during germination and fermentation process (Cosgrove and Irving, 1980).

4.4 Calculation of molar ratios of phytate: calcium, phytate: iron, phytate: zinc and (Calcium x Phytate):Zinc in fermented sorghum, maize, maize/soybean blend and sorghum/soybean blends flours.

The molar ratios of phytate and zinc, iron and calcium of diets to predict their bioavailability are shown in Table 4.8 and 4.9. Phytate:calcium molar ratio ranged between 1.21±0.12 and 0.07±0.00 for Diet 1 and Diet 3 respectively whereas phytate:calcium molar ratio ranged between 0.63±0.05 and 0.06±0.00 for Diet 2 and Diet 4 respectively (Table 4.8). The phytate:calcium molar ratio of Diet 3 (0.07±0.00) was significantly ($p<0.05$) lower than Diet 1 (1.21±0.12). Fermentation significantly ($p<0.05$) decreased the phytate:calcium molar ratio of Diet 1 from 1.21±0.12 to 0.06±0.00 and did not affect phytate:calcium molar ratio of Diet 3 after 72hrs of fermentation time.

Table 4.8: Relationship between phytate and bioavailability of selected minerals (calcium, iron and zinc) (molar ratio).

ANF: Mineral	Diet 1	Diet 2	Diet 3	Diet 4	*Critical values
Phytate: Calcium	1.21 ± 0.12^{c}	0.63 ± 0.05^{b}	0.07 ± 0.00^{a}	0.06 ± 0.00^{a}	>0.24
Phytate: Iron	7.62 ± 0.18^{b}	4.11 ± 1.45^{a}	4.57 ± 0.49^{a}	3.83 ± 0.00^{a}	>1.0
Phytate: Zinc	20.71 ± 0.23^{c}	10.94 ± 1.43^{b}	7.50 ± 0.03^{a}	7.41 ± 0.06^{a}	>15
(Ca)(Phytate): Zinc (mol/kg)	0.09 ± 0.00^{b}	0.05 ± 0.00^{a}	0.43 ± 0.00^{d}	0.34 ± 0.00^{c}	>0.5

Values are means of two duplicates ± SD. Means followed by the same letter in a row are not significantly different ($p<0.05$). Sample code as in Table 3.1

***Sources:** phytate:calcium >0.24 (Morris and Ellis, 1985), phytate: iron >1 (Hallberg *et al.*, 1987), phytate:zinc >15 (Adeyeye *et al.*, 2000); (Umeta *et al.*, 2005) and Ca x phytate:zinc >0.5 (Ellis *et al.*, 1987) and (Davies and Warrington, 1986).

Table 4.9: Relationship between phytate and bioavailability of selected minerals (Calcium, iron and zinc) (molar ratio).

ANF: Mineral	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	*Critical values
Phytate: Calcium	1.57±0.01^{b}	2.72±0.08^{c}	0.06±0.00^{a}	0.09±0.00^{a}	0.01±0.00^{a}	>0.24
Phytate: Iron	8.68±1.79^{d}	8.63±0.22^{d}	4.34±0.41^{b}	5.93±0.74^{c}	1.54±0.14^{a}	>1.0
Phytate: Zinc	14.92±3.18^{d}	20.78±1.25^{e}	6.34±0.11^{b}	10.74±0.78^{c}	1.75±0.16^{a}	>15
(Ca)(Phytate):Zinc (mol/kg)	0.03±0.00^{a}	0.02±0.00^{a}	0.34±0.00^{d}	0.31±0.02^{c}	0.20±0.00^{b}	>0.5

Values are means of two duplicates ± SD. Means followed by the same letter in a row are not significantly different ($p<0.05$). Sample code as in Table 3.1

***Sources:** phytate:calcium >0.24 (Morris and Ellis, 1985), phytate: iron >1 (Hallberg *et al.*, 1987), phytate:zinc >15 (Adeyeye *et al.*, 2000); (Umeta *et al.*, 2005) and Ca x phytate:zinc >0.5 (Ellis *et al.*, 1987) and (Davies and Warrington, 1986).

Phytate:calcium molar ratio ranged between 1.57±0.01 and 0.06±0.00 for Diet 5 and Diet 7 respectively whereas phytate: calcium molar ratio ranged between 2.72±0.08 and 0.09±0.00 for Diet 6 and Diet 8 respectively (Table 4.9). Phytate:calcium molar ratio of Diet 7 (0.06±0.00) was significantly ($p<0.05$) lower than that of Diet 5 (1.57±0.01). Fermentation significantly ($p<0.05$) increased phytate:calcium molar ratio of Diet 5 from 1.57±0.01 to 2.72±0.08 and did not affect phytate:calcium molar ratio of Diet 7 after 72hrs of fermentation time (Table 4.9). Phytate: calcium molar ratios >0.24, indicative of poor calcium available (Morris and Ellis, 1985) were found in Diet 1, Diet 2, Diet 5 and Diet 6 with value 1.21, 0.63, 1.57 and 2.72 respectively whereas high calcium availability was observed in Diet 3 (0.07), Diet 4 (0.06), Diet 7 (0.06), Diet 8 (0.09) and Diet 9 (0.01) analyzed (Table 4.8 and 4.9).

Phytate:iron molar ratio ranged between 7.62±0.18 and 4.57±0.49 for Diet 1 and Diet 3 respectively whereas Phytate:iron molar ratio ranged between 4.11±1.45 and 3.83±0.00 for Diet 2 and Diet 4 respectively (Table 4.8). The phytate:iron molar ratio of Diet 3 (4.57±0.49) was significantly ($p<0.05$) lower than the Diet 1 with value 7.62±0.18. Fermentation significantly ($p<0.05$) decreased the phytate: iron molar ratio of Diet 1 from 7.62±0.18 to 4.11±1.45 and did not affect phytate:iron molar ratio of Diet 3 after 72hrs of fermentation time.

Phytate: iron molar ratio ranged between 8.68±1.79 and 4.34±0.41 for Diet 5 and Diet 7 respectively whereas phytate: iron molar ratio ranged between 8.63±0.22 and 5.93±0.74 for Diet 6 and Diet 8 respectively (Table 4.9). Phytate:iron molar ratio of Diet 7 (4.34±0.41) was significantly ($p<0.05$) lower than Diet 5 (8.68±1.79). Fermentation significantly ($p<0.05$) increased phytate:iron molar ratio of Diet 7 from 4.34±0.41 to 5.93±0.74 and did not affect phytate:iron molar ratio of Diet 5 after 72hrs of fermentation time (Table 4.9). This resulted in phytate:iron molar ratios >1 which is regarded as indicative of poor bioavailability of iron (Hallberg *et al.*, 1987) in all diets (Table 4.8 and 4.9). This could be due to high phytate level in these foods.

Phytate:zinc molar ratio ranged between 20.71±0.23 and 7.50±0.03 for Diet 1 and Diet 3 respectively whereas Phytate:zinc molar ratio ranged between 10.94±1.43 and 7.41±0.06 for Diet 2 and Diet 4 respectively (Table 4.8). Phytate:zinc molar ratio of Diet 3 (7.50±0.03) was significantly ($p<0.05$) lower than Diet 1 (20.71±0.23). Fermentation significantly ($p<0.05$) decreased phytate:zinc molar ratio of Diet 1 from 20.71±0.23 to 10.94±1.43 and did not affect phytate:zinc molar ratio of Diet 3 after 72hrs fermentation time.

Phytate:zinc molar ratio ranged between 14.92±3.18 and 6.34±0.11 for Diet 5 and Diet 7 respectively whereas phytate:zinc molar ratio ranged between 20.78±1.25, 10.74±0.78 and 1.75±0.16 for Diet 6, Diet 8 and Diet 9 respectively (Table 4.9). Phytate:zinc molar ratio of Diet 7 (6.34±0.11) was significantly ($p<0.05$) lower than that of Diet 5 (14.92±3.18). Fermentation significantly ($p<0.05$) increased the phytate:zinc molar ratio of both Diet 5 and Diet 7 from 14.92±3.18 to 20.78±1.25 and from 6.34±0.11 to 10.74±0.78 respectively after 72hrs of fermentation time (Table 4.9). Phytate:zinc molar ratios >15 (critical value) indicative poor bioavailability of zinc (Adeyeye *et al.*, 2000); (Umeta *et al.*, 2005) were found in Diet 1 (20.71) and Diet 6 (20.78) whereas high zinc availability is observed in Diet 2 (10.94), Diet 3 (7.50), Diet 4 (7.41), Diet 5 (14.92), Diet 7 (6.34), Diet 8 (10.74) and Diet 9 (1.75) (Table 4.8 and 4.9).

Ca x phytate:zinc molar ratio ranged between 0.09±0.00 and 0.43±0.00 for Diet 1 and Diet 3 respectively whereas Ca x phytate:zinc molar ratio ranged between 0.05±0.00 and 0.34±0.00 for Diet 2 and Diet 4 respectively (Table 4.8). Ca x phytate:zinc molar ratio of Diet 3 (0.43±0.00) was significantly ($p<0.05$) higher than Diet 1 (0.09±0.00). Fermentation significantly ($p<0.05$) decreased Ca x phytate:zinc molar ratio of Diet 1 from 0.09±0.00 to 0.05±0.00 and decreased Ca x phytate:zinc molar ratio of Diet 3 from 0.43±0.00 to 0.34±0.00 after 72hrs of fermentation time.

Ca x phytate:zinc molar ratio ranged between 0.03±0.00 and 0.34±0.00 for Diet 5 and Diet 7 respectively whereas Ca x phytate:zinc molar ratio ranged between 0.02±0.00, 0.31±0.02 and 0.20±0.00 for Diet 6, Diet 8 and Diet 9 respectively (Table 4.9). Ca x phytate:zinc molar ratio of Diet 7 (0.34±0.00) was significantly ($p<0.05$) higher than Diet 5 (0.03±0.00). Fermentation significantly ($p<0.05$) decreased Ca x phytate:zinc molar ratio of Diet 7 from 0.34±0.00 to 0.31±0.02 and did not affect Ca x phytate:zinc molar ratio of Diet 5 after 72hrs of fermentation time (Table 4.9).

Ellis *et al.*(1987), and Davies and Warrington (1986) indicated that the ratio of Ca x phytate:zinc is a better predictor of zinc availability and that if the values were greater than 0.5 mol/kg, there would be interferences with availability of zinc. For all diets the Ca x phytate:zinc molar ratio is less than 0.5 which mean that there would not be interferences in availability of zinc. Akwaowo *et al.* (2000) reported that high levels of anti-nutrients such as oxalate and phytic acid are known to be very poisonous to humans. Since the results indicated that diets has low amount of phytates, so the bioavailability of essential dietary minerals such as calcium and zinc were assured.

It was observed in this study that the bioavailability of zinc and calcium in fermented diets was significantly ($p<0.05$) higher when compared with the unfermented diets after 72hrs of fermentation time. These are due to fermentation which enhances bioavailability of minerals by degrading phytate with microbial and native phytases that entangle macro- and trace- elements. The unity of this study is accord with those of fermented rice-dehulled blackgram blends (Sharma and Khetarpaul, 1997) and fermented cereal-based complementary foods (Urga and Narasimha, 1998). These studies indicated that fermentation hydrolyzed antinutrients from their organic bonds to increase mineral bioavailability.

It is well known that zinc, iron and calcium are essential trace elements for human nutrition (Kono and Yoshida, 1989). Children are more vulnerable to zinc, iron and calcium deficiency that adverse the growth rate and cognitive development (Hambidge *et al.*, 1985), presumably because of their low zinc, iron and calcium requirements for growth (Kono and Yoshida, 1989). The importance of foods as a source of dietary zinc, iron and calcium depends upon both the contents of these minerals in food products and the level of other constituents in the diet that affect their bioavailability. Phytic acid may reduce the bioavailability of dietary zinc, iron and calcium by forming insoluble mineral chelate at physiological pH.

Children in Ethiopia are especially very prone to deficiencies of mineral and trace elements, as they eat from the family dish and often cannot meet their specific nutrient needs. This is supported by Umeta *et al.* (2005), who showed that supplementation with zinc increased the linear growth of infants, particularly those who were stunted. Hence, phytate:zinc molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone (Urga and Narasimha, 1998); (Adeyeye *et al.*, 2000). The lower phytate: mineral ratios from fermented diets may be partly ascribed to the decreased content of phytic acid during fermentation which had a significant negative correlation ($p<0.05$) with the phytate: mineral ratio (bioavailability of minerals).

4.5 Physicochemical properties of fermented sorghum, maize, sorghum/soybean blend and maize/soybean blend flours.

4.5.1 pH and Titratable acidity

Changes in pH and total titratable acidity (TTA) of diets are shown in Figure 4.1 and 4.2 respectively. Fermentation was found to cause gradual reduction in pH value with increase in fermentation period. Fermentation significantly ($p<0.05$) decreased the pH from zero hrs to 72hrs resulted in pH drop from 6.41±0.01 to 3.91±0.00 and 6.42±0.00 to 4.11±0.007 for Diet 2 and Diet 4 respectively (Figure 4.1). Fermentation significantly ($p<0.05$) decreased the pH value of Diet 6 from 6.29±0.007 to 3.81±0.01 which is the least pH value obtained after 72hrs of fermentation time. The values are in agreement with the values reported on effect of fermentation and malting on the viscosity of maize/soybean weaning blends (Amankwah *et al.*, 2009a).

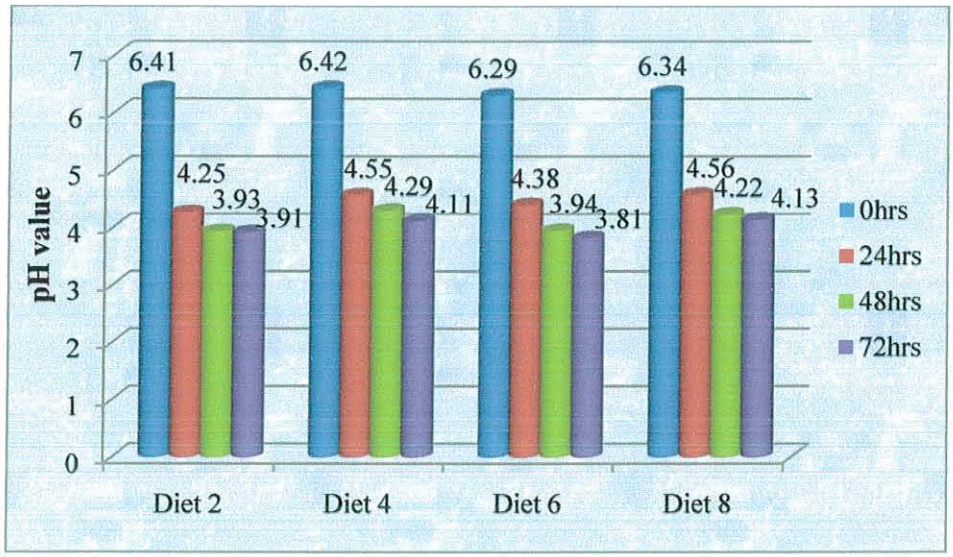


Figure 4.1: Change in pH of Diet 2, Diet 4, Diet 6 and Diet 8 during fermentation periods

Fermentation significantly ($p<0.05$) decreased the pH value of Diet 8 from 6.34±0.00 to 4.13±0.007 which are the highest pH values obtained after 72hrs of fermentation time (Figure 4.1) which is in agreement with the value reported from sensory evaluation, nutritional quality and antinutritional factors of traditionally co-fermented cereals/cowpea mixtures as infant complementary foods (Oyarekua, 2010). The total titratable acidity (TTA) of all Diets increased with fermentation period as can be seen from Figure 4.2. Diet 8 had the least titratable acidity (0.32) as shown in Figure 4.2 while Diet 4 had the highest titratable acidity (0.46) after 72hrs of fermentation time. These value are similar to the value reported on sensory evaluation, nutritional quality and antinutritional factors of traditionally co-fermented cereals/cowpea mixtures as infant complementary food (Oyarekua, 2010).

According to Akinrele (1970) the metabolic activities of microorganism during fermentation reduce the pH and increase titratable acidity. Mensah *et al.* (1991) reported that fermented foods with low pH have some antimicrobial activities and contributing to flavor of processed foods, exhibit longer shelf life of foods. Fermentation increased titratable acidity in all diets present in this study. A similar increase in acid production had been observed by Sefa-Dedeh *et al.* (2001) during the production of a weaning food from maize-cowpea blends. The increase in acidity is great significance as it was reported to reduce the incidence of diarrhea in infants consuming fermented maize porridge (Mensah *et al.*, 1990).

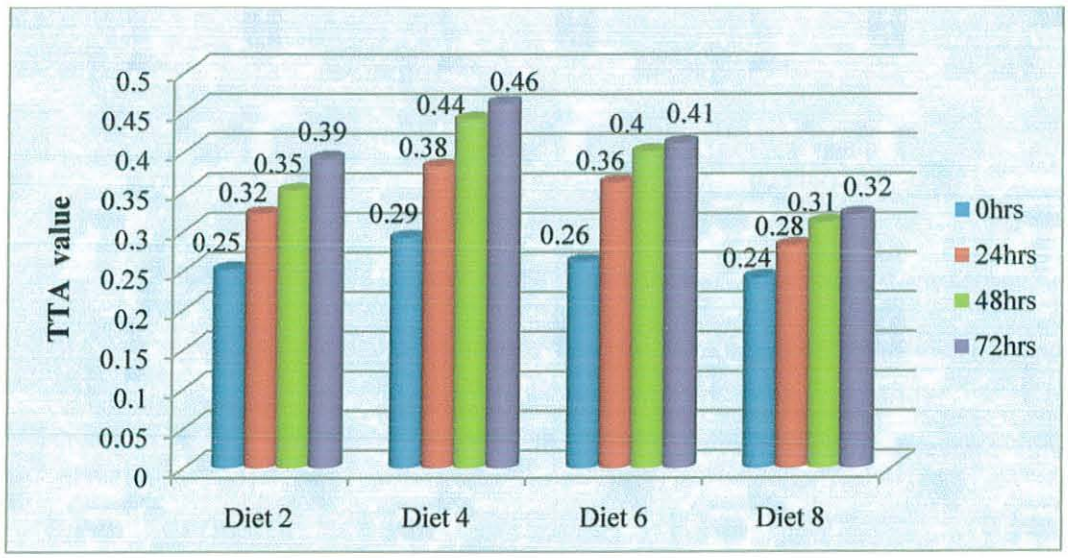
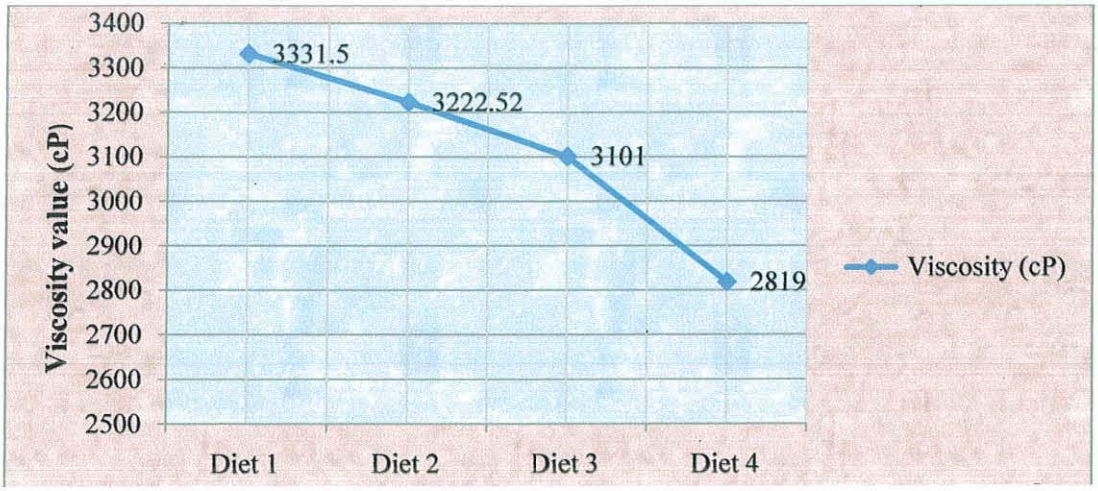


Figure 4.2: Change in total titratable acidity (TTA) of Diet 2, Diet 4, Diet 6 and Diet 8 during fermentation periods

4.5.2 Viscosity

The viscosity (gruels) value of Diet 1 and Diet 3 ranged between 3331.50±2.12cP and 3101.00±1.41cP respectively whereas viscosity of Diet 2 and 4 ranged between 3222.52±0.70cP and 2819.00±2.82cP respectively (Figure 4.3). Viscosity of Diet 3 with value 3101.00±1.4.1cP was significantly ($p<0.05$) lower than that of Diet 1 with value 3331.50±2.12cP. This is may be due to high fat content of the blend that contributed by soybean flour decreased the viscosity of diet. According to Plahar *et al.* (1997), in terms of starch stability, fortification with soy flour generally caused strengthening of starch granules and Griffith *et al.*(1998) reported that amylose, the starch component primarily responsible for gelatinization, formed insoluble complexes with lipids which reduced starch swelling capabilities upon heating. Therefore, a higher viscosity can be expected from blends with a lower fat content (Bekele, 2011)

Fermentation significantly ($p<0.05$) decreased the viscosity of Diet 3 from 3101.00±1.41cP to 2819.00±2.82cP and decreased the viscosity of Diet 1 from value of 3331.5±2.12cP to 3222.52±0.70cP after 72hrs of fermentation time (Figure 4.3). Previous studies have reported that a pH below 5.5 decreased the viscosity of the paste in cereal starch gruels (Nguyen *et al.*, 2007). The decrease in viscosity during fermentation may be ascribed due to increased amylase activity by taking the advantage of pH reduced medium. This enzyme can hydrolyze amylase and amylopectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges (Gibson *et al.*, 2006).



The viscosity values of Diet 5 and Diet 7 ranged between 3960.50±0.70cP and 2667±1.41cP respectively whereas the viscosity of Diet 6 and Diet 8 ranged between 3159±1.41cP and 2566±0.00cP respectively (Figure 4.4). The viscosity of Diet 7 with value 2667±1.41cP was significantly ($p<0.05$) lower than Diet 5 (3960.50±0.70cP). This is may be due to high fat content of the blend that contributed by soybean flour decreased the viscosity of diet. Fermentation was significantly ($p<0.05$) decreased the viscosity of Diet 7 from 2667±1.41cP to 2566±0.00cP and decreased the viscosity of Diet 5 from 3960.5±0.70cP to 3159±1.41cP after 72hrs of fermentation time (Figure 4.4).

Reduction in viscosity of fermented gruel could be due to a slightly greater breakdown of the paste during cooking, protein-starch interaction and amylose-lipid formation or due to differences in starch granules, size and composition of the fermented raw materials with the protein part contributing to giving lower paste viscosity. This agrees with the finding that supplementation of maize flour with bambara ground nut (Vigna subterranean L) reduced the peak viscosity of the product (Mbata *et al.*, 2009). The fermented gruels in this work could be desirable as infants' complementary food in terms of its low viscosity which by implication might be high in nutrients and energy density.

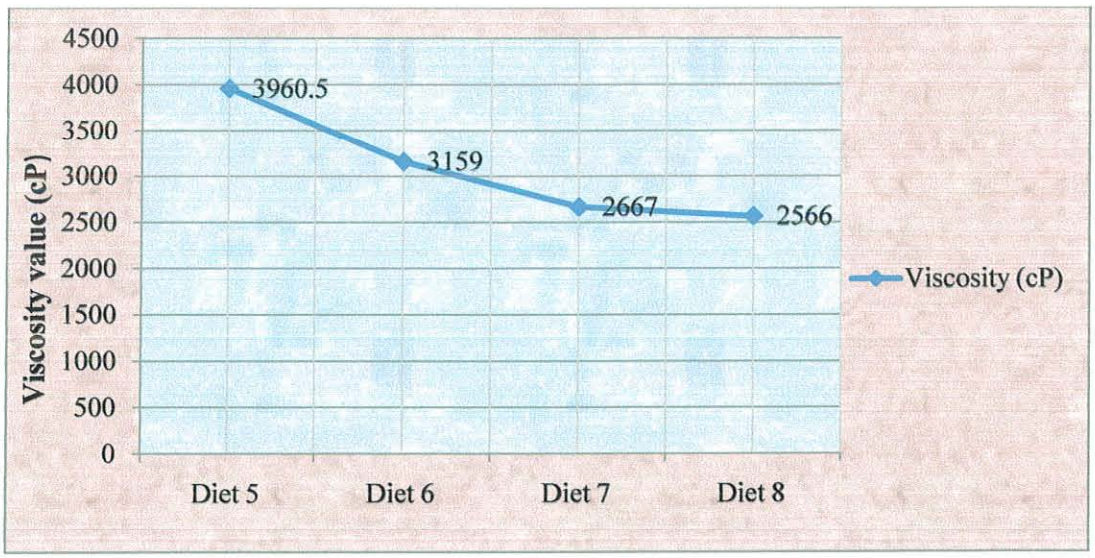


Figure 4.4: Viscosity of fermented maize (Diet 6) and maize/soybean blend (Diet 8) gruels measured at temperature of 45^0C.

4.6. Functional properties of fermented sorghum, maize, sorghum/soybean blend and maize/soybean blend flours.

The functional properties of food materials are very important for the appropriateness of the diet, especially for growing children. The consistency of energy density (energy per unit volume) and frequency of feeding are also important in determining the extent to which an individual will meet his or her energy and nutrient requirements (Omueti *et al.*, 2009b).

4.6.1 Bulk density

The decrease in bulk density of the fermented diet would be an advantage in the preparation of infant and children foods. Fermentation has been reported as a useful and traditional method for the preparation of low bulk complementary foods (Desikachar, 1980). In the present study the bulk density of Diet 1 and Diet 3 ranged between 0.86±0.04g/ml and 0.78±0.02g/ml respectively whereas the bulk density of Diet 2 and Diet 4 ranged between 0.76±0.00g/ml and 0.58±0.00g/ml respectively (Table 4.10). The bulk density of Diet 3 (0.78±0.02g/ml) was significantly ($p<0.05$) lower than Diet 1 (0.86±0.04g/ml). This may be due to the addition of soybean flour decreased bulk density of Diet 3 based complementary food. Fermentation significantly ($p<0.05$) decreased the bulk density of Diet 1 from 0.86±0.04g/ml to 0.76±0.00g/ml and decreased the bulk density value of Diet 3 from 0.78±0.02g/ml to 0.58±0.00g/ml after 72hrs of fermentation time (Table 4.10) which is in agreement with the value reported on weaning food from sorghum and Oil-seeds (Lalude and Fashakin, 2006).

Table 4.10: Bulk density, dispersibility, water and oil absorption of fermented sorghum and sorghum/soybean blend flours.

Sample code	Bulk density (g/ml)	Dispersibility (%)	Water absorption (g/g)	Oil absorption (g/g)
Diet 1	0.86 ± 0.04^{a}	76.0 ± 0.00^{c}	2.44 ± 0.01^{a}	3.67 ± 0.15^{a}
Diet 2	0.76 ± 0.00^{b}	80.5 ± 0.70^{d}	1.93 ± 0.06^{b}	3.95 ± 0.64^{b}
Diet 3	0.78 ± 0.02^{b}	73.5 ± 2.12^{b}	2.48 ± 0.02^{a}	4.18 ± 0.16^{b}
Diet 4	0.58 ± 0.00^{c}	70.0 ± 0.00^{a}	2.49 ± 0.04^{a}	4.69 ± 0.02^{c}

Values are means of two duplicates ± SD. Means followed by the same letter in a column are not significantly different ($p<0.05$). Sample code as in Table 3.1

The bulk density of Diet 5 and Diet 7 were ranged between 0.9±0.00g/ml and 0.78±0.02g/ml respectively whereas the bulk density of Diet 6, Diet 8 and Diet 9 ranged between 0.7±0.00g/ml, 0.72±0.02g/ml and 0.65±0.04g/ml respectively (Table 4.11). The bulk density of Diet 7 (0.78±0.02g/ml) was significantly ($p<0.05$) lower than that of Diet 5 (0.9±0.00g/ml).This is may be due to the addition of soybean flour decreased the bulk density of Diet 7 based complementary foods. Fermentation significantly ($p<0.05$) decreased bulk density of Diet 5 from 0.9±0.00g/ml to 0.7±0.00g/ml which is in agreement with the value reported on the determination of amino acid, fatty acid, mineral, functional and choking properties of germinated and fermented popcorn (Zea mayseverta) flour (Ijarotimi and Keshinro, 2011) and did not affect the bulk density of Diet 7 after 72hrs of fermentation time (Table 4.11).

Table 4.11: Bulk density, dispersibility, water and oil absorption of fermented maize and fermented maize/soybean blend flours.

Sample code	Bulk density (g/ml)	Dispersibility (%)	Water absorption (g/g)	Oil absorption (g/g)
Diet 5	0.90 ± 0.00^{a}	75.75 ± 0.35^{a}	2.15 ± 0.03^{a}	3.66 ± 0.16^{b}
Diet 6	0.70 ± 0.00^{b}	73.00 ± 0.00^{b}	2.35 ± 0.04^{b}	4.12 ± 0.00^{d}
Diet 7	0.78 ± 0.02^{c}	71.50 ± 0.70^{c}	2.47 ± 0.12^{c}	3.54 ± 0.07^{a}
Diet 8	0.72 ± 0.02^{bc}	68.50 ± 0.70^{d}	2.51 ± 0.05^{c}	3.78 ± 0.02^{c}
Diet 9	0.65±0.04	60.00±0.00	3.06±0.22	3.85±0.24

Values are means of two duplicates ± SD. Means followed by the same letter in a column are not significantly different ($p<0.05$). Sample code as in Table 3.1

The lower bulk density implies that less quantity of the food samples would be packaged in constant volume thereby ensuring an economical packaging. However, the packed bulk densities would ensure more quantities of food samples being packaged, but less economical. Nutritionally, loose bulk density promotes easy digestibility of food products, particularly among children with immature digestive system (Osundahunsi and Aworh, 2002). Therefore, low bulk densities of Diet 2, Diet 4, Diet 6 and Diet 8 therefore suggest that the diets could be more useful in complementary foods formulation when compared with other diets.

4.6.2 Dispersibility

The dispersibility of Diet 1 and 3 ranged between 76.0±0.00% and 73.5±2.12% respectively whereas the dispersibility of Diet 2 and Diet 4 ranged between 80.5±0.70% and 70.0±0.00% respectively (Table 4.10). Dispersibility of Diet 3 (73.5±2.12%) was significantly ($p<0.05$) lower than the dispersibility of Diet 1 (76.0±0.0%). Fermentation significantly ($p<0.05$) increased the dispersibility of Diet 1 from 76.0±0.0% to 80.5±0.70% and decreased the dispersibility of Diet 3 from 73.5±2.12% to 70.0±0.00% after 72hrs of fermentation time (Table 4.10). The dispersibility of Diet 5 and Diet 7 ranged between 75.75±0.35% and 71.50±0.70% respectively whereas the dispersibility of Diet 6 and Diet 8 ranged between 73.00±0.00% and 68.5±0.70% respectively (Table 4.11). The dispersibility of Diet 7 (71.50±0.70%) was significantly ($p<0.05$) lower than the dispersibility of Diet 5 (75.75±0.35%).

Fermentation significantly ($p<0.05$) decreased the dispersibility of Diet 5 and Diet 7 from 75.75±0.35% to 73.00±0.00% and from 71.50±0.70% to 68.5±0.70% respectively after 72hrs of fermentation time (Table 4.11). However, those values were higher than the values reported by Bekele (2011). The dispersibility of Diet 9 (soybean flour) ranged between 60.00±0.00% which has lower than the soybean flours (91.84%) and (95.1%) reported by Anuonye *et al.* (2007) and Lin *et al.* (1974) respectively and higher than the value (32.70%) reported on commercially sold soybean flours (Edema *et al.*, 2005).

4.6.3 Water absorption capacity

The water absorption of Diet 1 and Diet 3 ranged between 2.44±0.01g/g and 2.48±0.02g/g respectively whereas the water absorption of Diet 2 and Diet 4 ranged between 1.93±0.06g/g and 2.49±0.04g/g respectively (Table 4.10). The water absorption of Diet 3 (2.48±0.02g/g) was not significantly ($p<0.05$) different from the water absorption of Diet 1 (2.44±0.01g/g). Fermentation did not significantly ($p<0.05$) affect water absorption of Diet 3 and decreased water absorption of Diet 1 from 2.44±0.01g/g to 1.93±0.06g/g after 72hrs of fermentation time which is in agreement with the value reported by Singh *et al.* (2012). This value is also in agreement with the value reported on weaning food from sorghum and Oil-seeds (Lalude and Fashakin, 2006).

The reduction in water retention capacity may be related to with fermentation process. Ahmed *et al.* (1988) reported that fermentation decreased water adsorption capacity of sorghum meal. This may be attributed to degradation of starch during fermentation. Low water absorption capacity would be desired in order to decrease the microorganism of food that causes spoilage of diets (Giami and Bekebain, 1992). Hence the shelf-life of such product would be extended.

The water absorption of Diet 5 and Diet 7 ranged between 2.15±0.03g/g and 2.47±0.12g/g respectively whereas the water absorption of Diet 6, 8 and 9 ranged between 2.35±0.04g/g, 2.51±0.05g/g and 3.06±0.22g/g respectively (Table 4.11). The water absorption of Diet 7 (2.47±0.12g/g) was significantly ($p<0.05$) higher than that of Diet 5 (2.15±0.03g/g). This is may be due to the addition of soybean flour which increases the water absorption of Diet 7 based weaning food (Bekele, 2011).This was also similar to the observation by Edema *et al.* (2005) who reported that increased water absorption with increased soy flour fortification.

Fermentation significantly ($p<0.05$) increased water absorption of Diet 5 from 2.15±0.03g/g to 2.35±0.04g/g and did not affect the water absorption of Diet 7 after 72hrs of fermentation time (Table 4.11). However, these values are indicates increased water absorption capacity than those reported by Edema *et al.* (2005).Water absorption capacity of Diet 9 in this study was higher than those of Awassa soybean and Belessa variety (Yimer, 2008) is 2.71g/g and 2.55g/g respectively, (Sosulski and Fleming, 1977), 2.4g/g. Water absorption capacity of flour is a useful indicator of whether protein can be incorporated with aqueous food formulations, especially, those involving dough handling (Sobukola and Aboderin). Interactions of protein with water, is important to properties such as hydration, swelling power, solubility and gelation (Etudaiye *et al.*, 2009).The high water absorption capacity of flour suggests that it could be useful in soup formulations (Olaofe *et al.*, 1994).

4.6.4 Oil absorption capacity

The oil absorption capacity of Diet 1 and 3 ranged between 3.67±0.15g/g and 4.18±0.16g/g respectively whereas the oil absorption capacity of Diet 2 and 4 ranged between 3.95±0.64g/g and 4.69±0.02g/g respectively (Table 4.10). The oil absorption capacity of Diet 3 (4.18±0.16g/g) was significantly ($p<0.05$) higher than that of Diet 1 (3.67±0.15g/g). Fermentation significantly ($p<0.05$) increased the oil absorption capacity of both Diet 1 and Diet 3 from 3.67±0.15g/g to 3.95±0.64g/g and from 4.18±0.16g/g to 4.69±0.02g/g respectively after 72hrs of fermentation time. The higher oil-binding capacity of sorghum flour suggests that this flour would be useful in formulation of foods where an oil holding property is an important consideration.

The oil absorption capacity of Diet 5 and 7 ranged between 3.66±0.16g/g and 3.54±0.07g/g respectively whereas the oil absorption capacity of Diet 6, 8 and 9 ranged between 4.12±0.00g/g, 3.78±0.02g/g and 3.85±0.24g/g respectively (Table 4.11). Fermentation significantly ($p<0.05$) increased the oil absorption capacity of both Diet 5 and Diet 7 from 3.66±0.16g/g to 4.12±0.00g/g and from 3.54±0.07g/g to 3.78±0.02g/g respectively after 72hrs of fermentation time (Table 4.11) which is in agreement with the value reported by Bekele (2011). Similarly, the values obtained

from current study are higher than that of (1.22ml -2.23ml) reported by Muhsin (2009) on extrusion cooking of full-fat soy flour.

The oil absorption capacity of Diet 9 (3.85±0.24g/g) was higher than those of Awassa soybean and Belessa variety reported by Yimer (2008). Oil absorption capacity is important as oil acts as flavor retainer and gives soft texture to food improving mouth-feel (Akobundu, 2009); (Aremu *et al.*, 2006). Since the diets from current study had good oil absorption capacity, it suggests the presence of good lipophilic constituents and therefore may be suitable for production of foods (Aremu *et al.*, 2006).

4.7 Sensory attributes of fermented sorghum, maize, sorghum/soybean blend and maize/soybean blend gruels

The color score in Diet 1 and Diet 3 ranged between 8.00±1.22 and 7.00±1.22 respectively whereas the color score in Diet 2 and 4 ranged between 6.80±0.83 and 6.20±1.48 respectively (Table 4.12). The color rating of Diet 3 (7.00±1.22) was significantly ($p<0.05$) different from the color score of Diet 1 (8.00±1.22). In terms of color, the Diet 3 was less accepted than Diet 1 and this could be attributed to the fact that it contained soybean flour compare with Diet 1. Fermentation significantly ($p<0.05$) decreased the color score in Diet 1 and 3 from 8.00±1.22 to 6.80±0.83 and from 7.00±1.22 to 6.20±1.48 respectively after 72hrs of fermentation time (Table 4.12) which are in agreement with the score reported by Mihiret (2009). These results also similarly with those observed by Mihiret (2009) who reported that length fermentation period decreases the perceived characteristic of color, taste and overall acceptability of the products.

Table 4.12: Sensory evaluation of fermented sorghum and sorghum/soybean blend gruels

Sample code	Color	Taste	Aroma	Texture	Overall acceptability
Diet 1	8.00 ± 1.22^{c}	7.20 ± 2.90^{c}	5.80 ± 1.40^{b}	6.20 ± 0.83^{a}	6.80 ± 1.16^{b}
Diet 2	6.80 ± 0.83^{b}	6.00 ± 1.00^{a}	5.20 ± 1.30^{a}	6.80 ± 0.44^{b}	6.20 ± 0.83^{a}
Diet 3	7.00 ± 1.22^{b}	6.60 ± 2.77^{b}	7.11 ± 1.64^{c}	7.60 ± 0.54^{c}	7.00 ± 1.41^{b}
Diet 4	6.20 ± 1.48^{a}	6.01 ± 2.04^{a}	7.00 ± 1.74^{c}	8.40 ± 0.64^{d}	7.10 ± 1.42^{b}

Values are means of two duplicates ± SD. Means followed by the same letter in a column are not significantly different ($p<0.05$). Sample code as in Table 3.1

The color score in Diet 5 and Diet 7 ranged between 8.80±0.44 and 7.80±1.09 respectively whereas the color score in Diet 6 and 8 ranged between 7.80±0.43 and 7.26±1.14 respectively (Table 4.13). Color score in Diet 5 (8.80±0.44) was significantly ($p<0.05$) higher than Diet 6, 7 and 8 with scores 7.80±0.43, 7.80±1.09 and 7.26±1.14 respectively. In terms of color, the Diet 7 was less accepted than Diet 5 and this could be attributed to the fact that it contained soybean flour compare with Diet 5. Fermentation significantly ($p<0.05$) decreased the color score in Diet 5 and 7 from 8.80±0.44 to 7.80±0.43 and from 7.80±1.09 to 7.26±1.14 respectively after 72hrs of fermentation time (Table 4.13).

Table 4.13: Sensory evaluation of fermented maize and maize/soybean blend gruels

Sample code	Color	Taste	Aroma	Texture	Overall acceptability
Diet 5	8.80 ± 0.44^{c}	8.20 ± 0.83^{d}	5.40 ± 1.78^{a}	5.80 ± 1.11^{a}	6.87 ± 0.83^{b}
Diet 6	7.80 ± 0.43^{b}	5.40 ± 0.89^{a}	5.21 ± 1.77^{a}	6.60 ± 1.88^{b}	6.23 ± 0.83^{a}
Diet 7	7.80 ± 1.09^{b}	7.60 ± 0.81^{c}	6.20 ± 0.83^{b}	6.40 ± 1.81^{b}	7.00 ± 0.70^{b}
Diet 8	7.26 ± 1.14^{a}	6.70 ± 1.09^{b}	6.12 ± 1.48^{b}	7.20 ± 0.83^{c}	6.20 ± 0.83^{a}

Values are means of two duplicates ± SD. Means followed by the same letter in a column are not significantly different ($p<0.05$). Sample code as in Table 3.1

The taste score in Diet 1 and Diet 3 ranged between 7.20±2.90 and 6.60±2.77 respectively whereas the taste score in Diet 2 and 4 ranged between 6.00±1.00 and 6.01±2.04 respectively (Table 4.12). Taste score in Diet 1 (7.20±2.90) was significantly ($p<0.05$) higher than Diet 2, 3 and 4 with score 6.00±1.00, 6.60±2.77 and 6.01±2.04 respectively. Fermentation significantly ($p<0.05$) decreased the taste score in Diet 1 from 7.20±2.90 to 6.00±1.00 and decreased the taste score in Diet 3 from 6.60±2.77 to 6.01±2.04 after 72hrs of fermentation time.

The taste of Diet 2 and 4 were the least preferred by the panelist compare to Diet 1 and 3 due to higher acidity resulted from fermentation. According to the comment of panelists, the mouth feel of fermented gruel for 72hrs is sour and this comes from the acidic nature of the flour. A previous study on fermented sorghum indicated that the preference of the panelists for the characteristic taste and overall acceptability of the fermented gruels decreased with the increasing fermentation period (Mihiret, 2009). The present studies also show that the unfermented diets were rated significantly ($p<0.05$) higher in terms of taste and color compared to fermented diets.

The taste score in Diet 5 and Diet 7 ranged between 8.20±0.83 and 7.60±0.81 respectively whereas the taste score in Diet 6 and 8 ranged between 5.40±0.89 and 6.70±1.09 respectively. Taste score in Diet 3 and 7 were significantly ($p<0.05$) less preferred by the panelist than Diet 1 and 5 respectively. This could be due to the fact that the Diet 3 and 7 contained soybean flour that contains antinutritional compounds which are responsible for unpleasant flavor and bitterness that decreases the perceived characteristic of taste in diets (Muhsin, 2009). Fermentation significantly ($p<0.05$) decreased the taste score in Diet 5 and Diet 7 from 8.20±0.83 to 5.40±0.89 and from 7.60±0.81to 6.70±1.09 respectively after 72hrs of fermentation time (Table 4.13). This confirmed the observation made by Mensah *et al.* (1991) who reported that long hours of fermentation decrease sweetness of product and most mothers are less preferred to feed their infants porridges that were fermented beyond 24hrs.

The aroma score in Diet 1 and Diet 3 ranged between 5.80±1.40 and 7.11±1.64 respectively whereas the aroma score in Diet 2 and 4 ranged between 5.20±1.30 and 7.00±1.74 respectively (Table 4.12). The aroma score in Diet 3 (7.11±1.64) significantly ($p<0.05$) higher than that of Diet 1 and Diet 2 with scores 5.80±1.40 and 5.20±1.30 respectively. Fermentation significantly ($p<0.05$) decreased the score of aroma in Diet 1 from 5.80±1.40 to 5.20±1.30 and did not affect the score of aroma in Diet 3 after 72hrs of fermentation time. Therefore, the results and comments from the panelists imply that Diet 3 has better appetite for the infants. However, the aroma of Diet 2 was the least preferred by the panelist due to higher acidity resulted from fermentation time.

The aroma score in Diet 5 and Diet 7 ranged between 5.40±1.78 and 6.20±0.83 respectively whereas the aroma score in Diet 6 and 8 ranged between 5.21±1.77 and 6.12±1.48 respectively. The aroma score in Diet 7 (6.20±0.83) was significantly ($p<0.05$) higher than that of Diet 5 and Diet 6 with scores 5.40±1.78 and 5.21±1.77 respectively. Fermentation did not significantly ($p<0.05$) affect the aroma score in Diet 5 and Diet 7 after 72hrs of fermentation time (Table 4.13). The improved aroma score in diet 3 and 7 could be a result of supplementation of soybean flour.

The texture score in Diet 1 and Diet 3 ranged between 6.20±0.83 and 7.60±0.54 respectively whereas the texture score in Diet 2 and 4 ranged between 6.80±0.44 and 8.40±0.64 respectively (Table 4.12). The texture score in Diet 3 with score (7.60±0.54) was significantly ($p<0.05$) higher than the texture of Diet 1 and Diet 2 with score of 6.20±0.83 and 6.80±0.44 respectively. Fermentation significantly ($p<0.05$) increased the score of texture in Diet 1 and Diet 3 from score 6.20±0.83 to 6.80±0.44 and from 7.60±0.54 to 8.40±0.64 respectively after 72hrs of fermentation time (Table 4.12).

The texture score in Diet 5 and Diet 7 ranged between 5.80±1.11 and 6.4±1.81 respectively whereas the texture score in Diet 6 and 8 ranged between 6.60±1.88 and 7.20±0.83 respectively (Table 4.13). The texture score in Diet 7 (6.4±1.81) was significantly ($p<0.05$) higher than the texture of Diet 5 with scores (5.80±1.11). These results similarly with those observed by Omueti *et al.* (2009b) who reported that fortified maize with soy flour, groundnut and crayfish was more acceptable than unfortified yellow maize in terms of texture but not in color. Fermentation significantly ($p<0.05$) increased the texture score in Diet 5 and 7 from 5.80±1.11 to 6.60±1.88 and from 6.40±1.81 to 7.20±0.83 respectively after 72hrs of fermentation time (Table 4.13).

The overall acceptability score in Diet 1 and 3 ranged between 6.80±1.16 and 7.00±1.41 respectively whereas the overall acceptability score in Diet 2 and 4 ranged between 6.20±0.83 and 7.10±1.42 respectively (Table 4.12). Overall acceptability of Diet 3 (7.00±1.41) was not significantly ($p<0.05$) different from Diet 1. Fermentation significantly ($p<0.05$) decreased score of overall acceptability in Diet 1 from 6.80±1.16 to 6.20±0.83 and did not affect the score of overall acceptability in Diet 3 after 72hrs of fermentation time (Table 4.12). These results contrary with those observed by Oyewole and Ogundele (2004) who reported that the microbial population increased with increase in period of fermentation contributing significantly to the overall acceptability of cassava.

The overall acceptability score in Diet 5 and 7 ranged between 6.87±0.83 and 7.00±0.70 respectively whereas the overall acceptability score in Diet 6 and 8 ranged between 6.23±0.83 and 6.20±0.83 respectively (Table 4.13). The overall acceptability score in Diet 7 (7.00±0.70) was not significantly ($p<0.05$) different from the score in Diet 5 with scores (6.87±0.83). Fermentation significantly ($p<0.05$) decreased the score of overall acceptability in Diet 5 and 7 from 6.87±0.83 to 6.23±0.83 and from 7.00±0.70 to 6.20±0.83 respectively after 72hrs of fermentation time (Table 4.13). The preference of the panelists for the characteristic color, taste, aroma, texture and overall acceptability of formulation diets were above average likeable by panelists which are in agreement with those reported by Ugwuona *et al.* (2012) who stated that the samples were generally accepted by the consumers when sensory attributes had average mean score higher than the mean mark (4.5) of the 9-point scale. Therefore, the diets were generally accepted by the consumers.

4.8 Selection criteria for determining optimal fermented sorghum/soybean blend and maize/soybean blend flours

A ranking system using three nutritional criteria, i.e., Protein content, energy value and sensory attributes, was devised to determine the optimal blend combination according to the method modified by Griffith *et al.* (1998) (Table 4.14). Based on the relative importance and interrelationship of those criteria, ranking was reported on an equal weight basis. The weighting of those criteria as to relative importance produced identical conclusive results. The four blends were ranked from 1 to 4 objectively determine the choice of complementary foods. The complementary food yielding the lowest score was considered to possess the most suitable nutritional characteristics. As can be observed from Table 4.14 Fermented sorghum/soybean blend had lowest ranking score followed by unfermented sorghum/soybean, unfermented maize/soybean and fermented maize/soybean blends diets respectively. Therefore, fermented sorghum/soybean blend diet was concluded to possess the most desirable nutritional profile among formulated food samples.

Table 4.14: Ranking of fermented sorghum/soybean blend and maize/soybean blend foods to determine optimal nutritional profile

Parameters	Protein (g/100g)	Energy (kcal)	Sensory attributes	Total score
Unfermented sorghum/soybean	3	1	3	7
Fermented sorghum/soybean	1	3	1	5
Unfermented maize/soybean	4	2	2	8
Fermented maize/soybean	2	4	4	10

Sample code as in Table 3.1

CHAPTER FIVE

Conclusions and recommendation

5.1 Conclusions

In conclusion, this research shows that nutritious, acceptable and affordable complementary foods can be formulated using our locally available food items that can be better than traditional complementary foods. In the present work, it was concluded that enriching sorghum and maize with soybean increased the protein, energy, fat, ash, fibre and minerals contents of sorghum and maize-based complementary foods. Natural fermentation is more acceptable process as it is inexpensive, simple, easily understood and culturally acceptable where due to economic reasons, access to animal sources of protein is minimal by which people can obtain good quality food and this process can performed at their own homes. In the present work, it was observed that natural fermentation process significantly improves nutritional value of formulated foods by reducing antinutrients. It is also possible to conclude from study that fermentation process change protein and energy value of diets.

After 72hrs of fermentation time the protein content increased in all diets and a composite of fermented sorghum/soybean flour had high protein content compare to other diets. Fermentation decreased the total ash and crude fat content of sorghum/soybean and maize/soybean composite flours whereas the carbohydrate content of both composite flours was increased. Fermentation increased crude fibre and moisture content of composite sorghum/soybean flour and did not affect the crude fibre and moisture content in maize/soybean composite flour.

Fermentation also decreased the minerals content of sorghum/soybean and maize/soybean composite flours. On the other hand, gruels prepared from fermented blend flours were less viscous and dietary bulkiness nature due to the fermentation effect. This obviously will increased food intake of many infants. Fermentation significantly changes the sensory properties of diets. Fermented sorghum/soybean blend flour sample was concluded to possess the most desirable nutritional profile among the formulated foods followed by sorghum/soybean, maize/soybean and fermented maize/soybean blends diet respectively.

5.2 Recommendations

During the process of undergoing this research paper, there had been some constraints and results. Based on this study, the following recommendations are made.

- The study has demonstrated the effects of natural fermentation that can significantly reduce phytic acid and tannin content of weaning blends and enhance acceptability of product. It is recommended that animal feed experiment be carried out to evaluate the effects of these processing steps on nutritional quality of fermented weaning blends to further check and compare with the results of this thesis work.

- Many fermented foods are made from mixtures of legumes and cereals which have great nutritional advantage. This may be recommended that other researches should conduct to analysis amino acids profile, vitamins and other antinutrients.

- It is also recommended for researches to conduct other functional and physico-chemical properties should be conducted.

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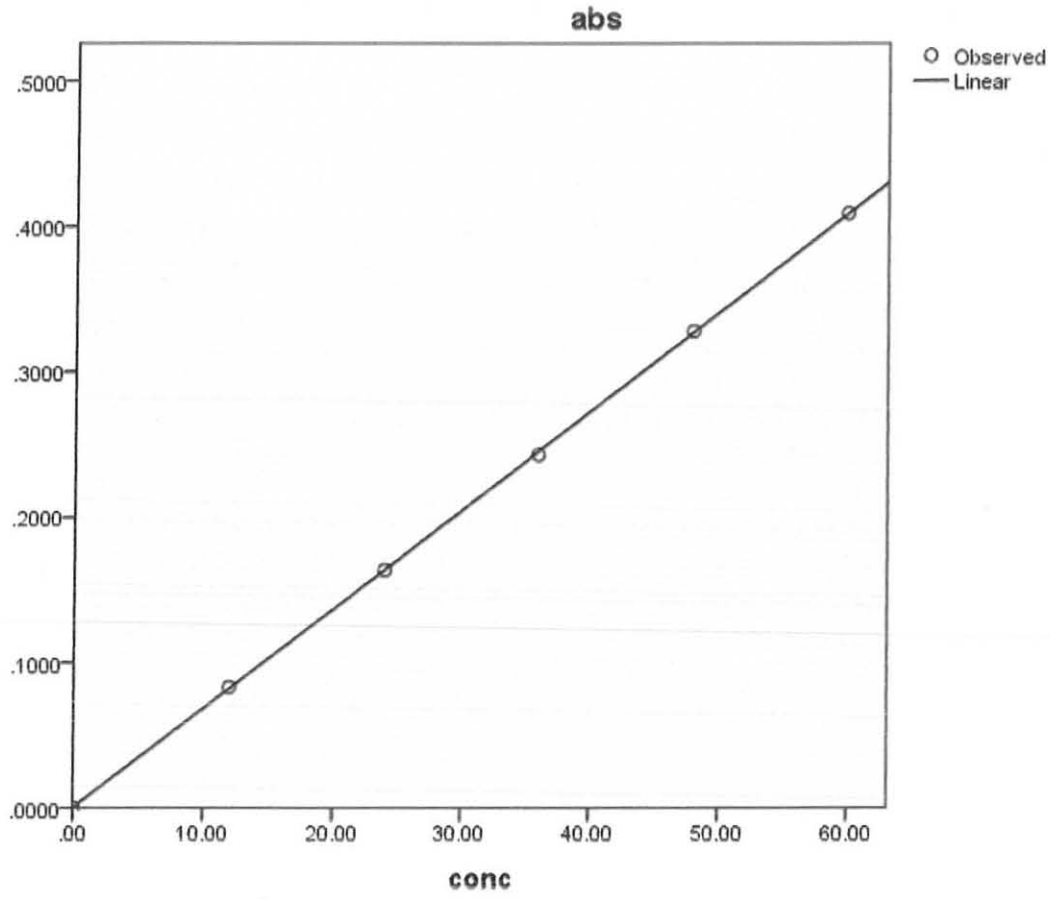

Appendix 3: Standard Curve for Tannin Determination (R^2 =0.999)