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ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
FOOD SCIENCE AND NUTRITION PROGRAM

**The Effect of Natural Fermentation on Some Antinutritional
Factors, Minerals, Proximate Composition and Sensory
Characteristics in Sorghum Based Weaning Food**

By Mihiret Kassa Alemu

*A Thesis Presented 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.*

June, 2009

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ACRONYMS

Ab	Absorbance
ANOVA	Analysis of Variance
AOAC	Official Methods of Analysis Association of Official Analytical Chemists
AAS	Atomic Absorption Spectrophotometer
CSA	Central Statistics Agency
cP	Centipoises
EHNRI	Ethiopian Health and Nutrition Research Institute
ESE	Ethiopian Seeds Enterprise
HED	High Energy Density
Insp ₃	Inositol Tri-phosphate
Insp ₄	Inositol Tetra-phosphate
Insp ₅	Inositol Penta-phosphate
Insp ₆	Inositol Hexa-phosphate
PEM	Protein Energy Malnutrition
R	Pearson Correlation Coefficient
RPM	Revolution per Minute
RVA	Rapid Viscoanalyzer
SD	Standard Deviation
SEM	Standard Error of the Mean
SPSS	Statistical Product and Service Solutions
TTA	Total Titratable Acidity

ABSTRACT

The effect of natural fermentation of two sorghum cultivars on antinutritional factors (tannin and phytate), minerals (Ca, Fe, Zn and P), proximate analysis and sensory characteristics were investigated. Flours from both cultivars were fermented at room temperature (20- 23⁰ C) for 0, 12, 24, 36 and 48h at a concentration of 1:3 dilutions (w/v). TTA and pH were determined immediately at the end of each fermentation period and the samples were dried at 70⁰ C in air oven drier for 36h. Fermentation caused an increase in the protein, TTA, energy content and improved mineral bioavailability and decrease in fat, carbohydrate, ash, crude fiber, viscosity, antinutritional factors, phytate: mineral molar ratios and pH of sorghum flour. The bioavailability of zinc (phytate: zinc molar ratio <15) after 24-48h fermentation of Gobiye cultivar was found to meet the critical limit. The bioavailability of calcium and iron was below the critical limit in all the samples analyzed; phytate: calcium molar ratio >0.24 and phytate: Fe molar ratio > 0.15. However calcium had no effect on the absorption of zinc ([Calcium x Phytate]: [Zinc] <0.5 millimolar). When the samples were subjected to sensory evaluation, there was no significant difference (p<0.05) in the appearance and aroma of the unfermented and gruel samples fermentation for 12h. The panelists however noted that appearance, aroma, texture, taste and overall acceptability of the gruel prepared from sorghum flour that was subjected to 48h fermentation differ from others and least acceptable. Taste and overall acceptability decreased significantly (p<0.05) along with period of fermentation. Ethiopian weaning mothers should be encouraged to prepare 12 to 24h fermented sorghum based weaning gruels that may blend with either legumes or milk powder by adding some amount of sugar. Further research on cereal based fermented weaning foods needs to be conducted in Ethiopia. Researchers should also take into consideration the incorporation of phytase enzymes into cereal plants to enhance the nutritional value in addition to yield improvement by the application of biotechnology.

1. INTRODUCTION

1.1. Background and justification

During the first six months of life, most infants obtain all the energy and nutrients they need from breast milk. By the age of six months, however, they need additional or complementary foods.

Several studies have reported that the complementary food given to infants by many mothers in developing countries, Ethiopia inclusive, are deficient in macronutrients (protein, carbohydrates and fat, leading to protein energy malnutrition), micronutrients (minerals and vitamins, leading to specific micronutrient deficiencies) or both (Ijarotimi, 2008).

At the age of six months, most infants show signs that they are ready to start other foods. They may have one or two teeth and begin chewing. Even though they are getting plenty of breast milk, they seem extremely hungry and reach out for the food their mothers are eating. If a mother does not start giving weaning foods at this stage, the child may stop gaining weight at a healthy rate and become underweight.

The type and quality of weaning food play a vital role in the growth and development of children. The high rates of malnutrition reported in cereal consuming areas of Africa reveal the bulkiness and high viscosity of the predominant cereal-based weaning foods (John *et al.*, 2008). Phytic acid in cereal-based complementary foods inhibits iron absorption. Low iron absorption from cereal porridges contributes to the high prevalence of malnutrition deficiency in infants from developing countries (Hurrell *et al.*, 2003).

To improve the nutrient intake of weaning-age children, several food preparation technologies have been advocated that effectively increase the nutritional value of weaning porridges and reduce the risk of infection from such foods. Non-alcoholic fermentation of cereals for a limited period of time has been found to improve the amino acid composition and vitamin content, increase protein and starch digestibility, increase the bioavailability of minerals, and to lower the levels of antinutrients; the fermented product is seldom free of micro-organisms (Badau *et al.*, 2006).

However, despite many results in the literature as to the viscosity-reducing effect of fermentation, this is rarely substantiated and there has been little work specifically addressing the effect of fermentation on viscosity and energy density. The findings are highly contradictory, and appear to vary according to the method of fermentation and particularly the microorganism responsible (Wambugu *et al.*, 2002). A limited decrease in viscosity of cereal gruels has also been observed after natural lactic acid fermentation of starchy raw material but additional treatments are needed to prepare high energy gruels (Guyot *et al.*, 2007).

1.2. Weaning Food

Weaning is a gradual withdrawal of feeding a baby with the mother's milk and start feeding it with solid food. The transition from milk to solid or adult food is a critical period in the life of a child as the weaning practices by the mother profoundly determines the child's growth and development (Olorunfemi *et al.*, 2006). This period which could start from four to six months of age, varies from one socioeconomic status to the other. Weaning foods are increased in amount gradually so that eventually the child gets enough energy and nutrients from family

foods. The process is completed by the time a child reaches two to three years of age and no longer takes breast milk.

The large numbers of studies are of nutritional disorders like marasmus and kwashiorkor due to protein energy malnutrition, observed during weaning period can be reduced through the use of nutritionally balanced home produced weaning foods (Olorunfemi *et al.*, 2006).

In most African countries, traditional weaning foods are based on local starchy staples usually fermented, ground cereals that are processed into porridges such as Ogi. Sorghum has been widely used to produce Ogi which is a popular weaning food in Nigeria (Olorunfemi *et al.*, 2006).

Poor child feeding practices of low-income families is responsible for the high prevalence of Protein energy malnutrition among children in developing countries (Ijarotimi, 2008). In order to solve these nutritional problems, several efforts have been made to formulate weaning foods from local food materials; however, it is evident recently that there is heavy reliance on most of these food materials thereby leading to inaccessibility by many mothers as a result of the cost of the materials and mode of production processes (Ijarotimi, 2008). Thus most families have to depend on the local material and technology for the preparation of weaning foods.

One of the major nutritional problems faced by young children in the eastern and southern African region during the weaning period is that weaning foods (which are commonly prepared in the form of porridges) made from the major staple foods (mainly cereals and roots and tubers) have low nutrient density, mainly because they are high in unmodified starch and low in fat (Badau *et al.*, 2006). A young child must consume a large volume of this porridge if its energy and other nutrient requirements are to be met during the period when breast milk alone

is insufficient. Many children are unable to eat such quantities mainly by virtue of their small stomachs, resulting in insufficient intakes of energy, protein, and other nutrients (Badau *et al.*, 2006). The problem is compounded if children are fed infrequently (because of other demands on the mother's time) or if appetite is reduced because of illness.

During the weaning process, children are particularly vulnerable to malnutrition. The weaning period is a very critical period in promoting child nutrition and survival. The period would have been less problematic if only mothers know how to feed their children with locally available food materials. Studies indicated that the vast majority of children in developing countries are not only improperly fed but are also underfed (Akeredolu *et al.*, 2005). Prevalence of Protein Energy Malnutrition (PEM) in infants after six months of age is high in Africa (Lalude and Fashakin, 2006). This is because infants at this stage of development require higher energy and proteins in their diet so as to meet the increasing demand for metabolism.

1.3. Statement of the problem

In developing countries, because of limited access to animal products (meat, egg, fish) that provide high intakes of heme iron and zinc, the main dietary sources of iron and zinc are cereals and legumes (Abdel-Rhaman *et al.*, 2005). These cereals and legumes contain antinutritional factors that reduce the bioavailability of nutrients (Melaku *et al.*, 2005).

Similar to other foodstuffs certain nutritional inhibitors and toxic substances are associated with sorghum as well (FAO, 1995). These antinutrients significantly reduce the bioavailability of different nutrients and may lead to malnutrition (Makokha *et al.*, 2002). As a result of this; children are the one who are seriously affected. This is because their physiology is not well

adapted to digest more starchy and bulky foods and retrieve sufficient amount of nutrients (Wambugu *et al.*, 2002).

The development of low-cost, high-protein food supplements for weaning infants from local and readily available raw materials is a constant challenge for developing countries (Ali *et al.*, 2006). The availability of ready-made snacks and baby foods is limited in local markets and whatever items are available, they are expensive. Moreover, they are not available in remote areas (Thathola and Srivastava, 2002).

Recent data from the World Health Organization shown that about 60% of all deaths, occurring among children aged less than five years (under-five children) in developing countries, could partly be attributed to malnutrition (Faruque *et al.*, 2008). It has been estimated that nearly 50.6 million under-five children are malnourished, and almost 90% of these children are from developing countries (Faruque *et al.*, 2008).

Most weaning food technologies, e.g., drum drying and extrusion cooking, are either too complicated or too expensive for low-income families in developing countries. Thus, practical, low-technology processes for production of weaning foods with adequate paste viscosity and nutrient density are needed (Almeida-Dominguez *et al.*, 1993). Different methods have been tried to inactivate or detoxify the antinutritional factors in sorghum to improve their nutritional quality. Fermentation is one of the methods that result in a significant reduction in the antinutritional factor and improves the nutritional quality of the end product (Khetarpaul and Sharma, 1997).

2. OBJECTIVES

2.1. General objective

- To analyze the effect of natural fermentation on some antinutritional factors, minerals, proximate composition and sensory characteristics in sorghum based weaning food.

2.2. Specific Objectives

- ✓ To compare and contrast some minerals before and after natural fermentation of sorghum.
- ✓ To determine the effect of natural fermentation of sorghum on some antinutritional factors (phytate and tannin).
- ✓ To determine changes in pH and total titratable acidity of sorghum during fermentation.
- ✓ To determine the effect of natural fermentation on sensory attributes of sorghum based weaning gruel.
- ✓ To determine the effect of natural fermentation on proximate composition of sorghum cultivars.

3. LITERATURE REVIEW

3.1. Nutrition and child health

In developing countries, malnutrition persists as a principal health problem among children below the age of five (Fasasi *et al.*, 2007). Although the outcome of infant's weight at birth and its survival depends on the state of nutrition of the mother prior to conception and throughout gestation period, its nutrition in the first year of life has long-term consequences on the growth, brain development and the potential of the child in future (Ijarotimi, 2008).

The growth and survival of a child continue after birth by deriving its nutrition through breast feeding for the first six months of life. Afterwards, the breast milk tends to be inadequate to supply all the necessary nutrients that the child needs for the normal growth and cognitive development. This necessitated for the provisions of adequate weaning diets for the child. Infant-feeding practices constitute a major component of child caring practices apart from socio-cultural, economic and demographic factors. Somehow, these practices constitute one of the most neglected determinants of young child malnutrition in spite of their important role in growth pattern of children.

Recent studies have recognized the link between malnutrition and child feeding practices which showed that high numbers of children in developing countries are malnourished, and this has resulted in stunted growth, retardation in cognitive development, increase in morbidity and mortality rate (Ijarotimi, 2008).

Protein energy malnutrition usually manifests early, in children between six months and two years of age and is associated with early weaning, delayed introduction of complementary

foods, a low-protein diet and severe or frequent infections (Thathola and Srivastava, 2002). The major causes of protein energy malnutrition among infants in developing countries have been traced to low quantity and poor quality protein intake. These problems have been attributed to poverty and lack of nutritional knowledge among mothers. Several studies have shown that protein deficiency is a major nutritional problem among children; and has hindered their health, mental capability, school performance and productivity, thus affecting the country's economic growth (Thathola and Srivastava, 2002).

During the weaning period (4-6months) exclusive breast-feeding is recommended. Too early introduction of any other liquid or solid food has been shown in a wide variety of socioeconomic conditions, to reduce breast-milk production and increase risk of infection (WHO, 2008). WHO recommends that children should continue to be breast-fed up to two years of age or beyond, while receiving nutritionally adequate and safe complementary foods (WHO, 2008). Infants and children up to 2 years of age, especially vulnerable groups in general in almost every country and in particular in developing countries (WHO, 2008).

3.2. Malnutrition in Ethiopian children

Ethiopia is one of the countries in the Sub-Saharan Africa with the highest rates of malnutrition in children. Malnutrition results from the interaction between poor diet and disease and leads to most of the anthropometric deficits observed among children in Ethiopia (Zewditu *et al.*, 2001). Complementary foods are not introduced in a timely fashion for many children. At 6-8 months, only one in two children is receiving complementary foods. The use of a bottle with a nipple is not widespread in Ethiopia (CSA and Macro, 2006).

More than half of Ethiopian children ages 6-59 months are classified as anaemic, with 21 percent mildly anaemic, 28 percent moderately anaemic, and 4 percent severely anaemic (CSA and Macro, 2006).

The level of malnutrition is significant with nearly one in two (47 percent) Ethiopian children under five years of age stunted (short for their age), 11 percent wasted (thin for their height), and 38 percent underweight. In general, rural children and children of uneducated mothers are more likely to be stunted, wasted, or underweight than other children (CSA and Macro, 2006).

Regional variation in nutritional status of children is substantial. Stunting levels are above the national average in Amhara and SNNP. Wasting is higher than the national average in Somali, Benishangul-Gumuz, Amhara, Tigray and Dire Dawa. The percentage of underweight children is above the national average in Somali, Amhara, Tigray and Benishangul-Gumuz (CSA and Macro, 2006).

3.3. Sorghum

Sorghum is an important food crop in the semi-arid regions of Ethiopia. Because it is less dependent on rainfall, and therefore, less affected by fluctuations in environmental conditions. Still, there is a need to increase its production and adoption both in domestic use and in industrial technologies for bread and for infant foods (Asfaw *et al.*, 2005).

Sorghum is a genus with many species and subspecies, and there are several types of sorghum, including grain sorghum, grass sorghum (for pasture and hay), sweet sorghum (for syrups), and Broomcorn. Grain sorghum and maize (corn) are comparable in production, costs and in nutrition; therefore the growing environment is the largest determining factor for choosing

which crop to grow. Grain sorghum requires less water than corn, so is likely to be grown as a replacement to corn and produce better yields than corn in hotter and drier areas, such as Southern US, Africa, Central America and South Asia (Wikipedia contributors, 2009).

Determining when and where sorghum was domesticated has been a quandary for historians. Whether it was domesticated in Africa, or transported from Africa and domesticated in India then returned to Africa, is not certain (Wikipedia contributors, 2009). It is believed that African slaves took sorghum seeds with them to the US, and that is how it was introduced to what is now the #1 sorghum growing country (Wikipedia contributors, 2009).

Although breeding has resulted in better nutritional value of sorghum and better flavor, earlier sorghum crops had higher tannin levels, which caused offensive flavor and was advantageously used as a deterrent to birds. High-tannin sorghums are still grown where birds could cause significant losses (Asfaw *et al.*, 2005; Wikipedia contributors, 2009).

Sorghum, like many cereal grains, has a diversity of uses, including human consumption and animal feed. Sorghum is used for human nutrition and acts as the most important food crop in the world, following wheat, rice, maize and barley (FAO, 1995; Asfaw *et al.*, 2005). Globally, over half of all sorghum produced is used for human consumption (Wikipedia contributors, 2009). It is a major crop for many poor farmers, especially in Africa, Central America, and South Asia. Grain sorghum is used for flours, porridges and side dishes, malted and distilled beverages, and specialty foods such as popped grain. A more recent use of sorghum is for ethanol production (Wikipedia contributors, 2009).

Unfortunately, sorghum has low nutritional value and inferior organoleptic qualities due to the presence of antinutritional factors which make complexes with food ingredients (Makokha *et*

al., 2002). In addition, *in vivo* and *in vitro* studies indicate that the proteins of wet cooked sorghum are significantly less digestible than the proteins of other similar cooked cereals such as wheat and maize (Abdelhaleem *et al.*, 2008).

The antinutritional effect of tannin and phytate in sorghum has been demonstrated by many researchers (Ikemefuna and Atii, 1990; Makokha *et al.*, 2002; Abdelhaleem *et al.*, 2008). The tannin-protein interaction in sorghum involves hydrogen bonding and hydrophobic interactions (Abdelhaleem *et al.*, 2008). Sorghum prolamins (proline-rich proteins) bind strongly to sorghum tannins and these results in reduced protein digestibility (Abdelhaleem *et al.*, 2008). The phytate molecule, containing six phosphate groups, is highly charged. This makes it an excellent chelator and it can form insoluble complexes with proteins leading to reduced digestibility (Makokha *et al.*, 2002).

Sorghum is a staple food in many African countries and it contains a reasonable amount of protein (7.5–10.8%), ash (1.2–1.8%), oil (3.4–3.5%), fiber (2.3–2.7%) and carbohydrate (71.4–80.7) with a dry matter ranged from 89.2% to 95.3% depending on the type of cultivar (Idris *et al.*, 2007). Further, sorghum flour contained 11.0–13.0, 285–310 and 4.0–5.5 mg per 100 g Ca, P and Fe, respectively (Idris *et al.*, 2007).

Sorghum is rich in mineral content but its nutritional quality is dictated mainly by its chemical composition; presence of considerable amounts of anti-nutritional factors such as tannin, phytic acid, polyphenol and trypsin inhibitors that are undesirable (Gilani *et al.*, 2005). Hence, elimination or inactivation of such anti-nutritional compounds is absolutely necessary to improve the nutritional quality of sorghum, and effectively utilize its full potential as human

food (Idris *et al.*, 2005; Idris *et al.*, 2007), by using simple household technologies like fermentation (Ikemefuna and Atii, 1990; Makokha *et al.*, 2002; Abdelhaleem *et al.*, 2008).

An adequate mineral absorption is important especially for infants, children, elderly people and people in clinical situation (Idris *et al.*, 2007). It is evident that the nutritional importance of a given food/feed stuff depends not only on nutrient composition of raw foodstuff but also on the amount utilized (Idris *et al.*, 2007).

The methods employed to improve the nutritional quality and organoleptic properties of cereal-based foods include genetic improvement, amino acid fortification, supplementation or complementation with protein-rich sources and processing technologies which include milling, malting, fermentation or sprouting (Ugwu and Oranye, 2006).

Most of the agricultural researches on sorghum and other cereals have been related to varietal selection. The criteria for selection have always been resistance to disease or high yields, but nutritive quality has received less attention (Muzquiz *et al.*, 1999). A study of the composition and nutritive quality of cereals would therefore be of great interest, because the knowledge provided would also help to orient the work of investigators involved in varietal selection (Muzquiz *et al.*, 1999).

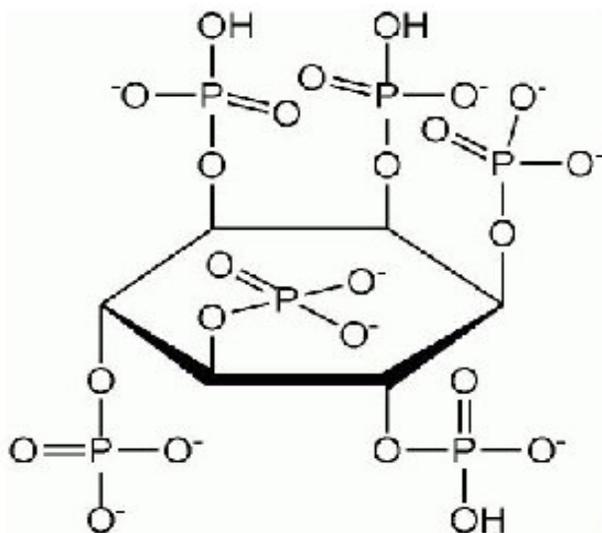
3.4. Antinutritional factors

As with other foodstuffs, certain nutritional inhibitors and toxic substances are associated with sorghum grains as well. Antinutritional factors can be classified broadly as those naturally present in the grains and those due to contamination which may be of fungal origin or may be related to soil and other environmental influences (FAO, 1995). These factors modify the

nutritional value of the individual grains, and some of them have very serious consequences on health of the consumers (FAO, 1995).

Similarly, the presence of high levels of tannins in cereals, such as sorghum, can result in significantly reduced protein and amino acid digestibility (Gilani *et al.*, 2005). However, they could be eliminated or reduced by processes such as soaking, dehulling, germination and fermentation (Sandberg, 2002; Ugwu and Oranye, 2006). The utilization of sorghum, being a cheap protein source, would be greatly enhanced if the most effective method of elimination of its toxic components is discovered (Ugwu and Oranye, 2006). Antinutritional factors lower the nutritional value of a food by lowering the digestibility or bioavailability of nutrients (Sandberg, 2002).

3.4.1. Phytates



Structure of Phytate (Insp_6), empirical formula= $\text{C}_6\text{P}_6\text{O}_{24}\text{H}_{18}$

Phytic acid [myoinositol 1, 2, 3, 4, 5, 6-hexakis (dihydrogen phosphate)], present in most plant foodstuffs as the phytate salt or a complex with protein, chelates with certain metal ions (such

as, calcium, zinc, copper, and iron) to form insoluble protein-mineral-phytate complexes (Khetarpaul and Sharma, 1997). These complexes fail to break down readily and make the minerals, especially divalent cations unavailable (Khetarpaul and Sharma, 1997).

Phytate (inositol hexaphosphate) constitutes 1–3% of cereal grains, legume seeds and nuts, and also occurs in low concentrations in roots, tubers and vegetables (Sandberg, 2002). Analysis of several varieties of sorghum indicated that the whole grain phytin phosphorus ranged from 170 to 380 mg per 100 g; over 85 percent of the total phosphorus in the whole grain bound as phytin phosphorus (FAO, 1995; Makokha *et al.*, 2002; Lorenz, 2007). Studies on the distribution of phytin phosphorus in sorghum grain also showed that a greater percentage of phytic acid was in the germ than in the bran and the least was in the endosperm (FAO, 1995). In particular, wholegrain cereals and legumes have a high content of phytate but also of the minerals Zn, Fe and Mg (Sandberg, 2002).

Phytate occurs as a mineral complex, which is insoluble at the physiological pH of the intestine. It is considered antinutritional, causing reduced uptake in the human intestine of essential dietary minerals such as Fe, Zn and Ca. A dose-dependent inhibition of Fe, Zn and Ca absorption by phytate has been demonstrated in humans (Sandberg and Andlid, 2002). Furthermore, it was found recently that inositol tri- and tetra- phosphate contribute to the negative effect on Fe absorption of processed food containing a mixture of inositol phosphates (Sandberg and Andlid, 2002), probably by interactions with the higher phosphorylated inositol phosphates.

Phytic acid binds trace elements and macro-elements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract and making dietary minerals unavailable for absorption and

utilization by the body (Sandberg, 2002; Melaku *et al.*, 2005). It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis (Muzquiz *et al.*, 1999). Moreover, the phosphorus in phytate has been considered to be largely unavailable to the organism because of the limited capacity of the organism's body to hydrolyse phytate in the small intestine (Muzquiz *et al.*, 1999).

Phytate represents a complex class of naturally occurring phosphorus compounds that can significantly influence the functional and nutritional properties of foods. Although the presence of these compounds has been known for over a century, their biological role is not completely understood (FAO, 1995). Phytic acid is the main phosphorus store in mature seeds. It renders also several minerals biologically unavailable to animals and humans. Bioavailability of iron in sorghum for human subjects was found to be affected more by phytin phosphorus than by tannin content of the grains (FAO, 1995).

Degradation of phytate can occur both during food processing and in the gastrointestinal tract. This degradation is of nutritional importance because it has been demonstrated that such controlled degradation improves the uptake of essential dietary minerals, i.e., Fe and Zn (Sandberg, 2002).

Major efforts are therefore made to reduce the amount of phytate in foods by means of phytate-degrading enzymes, phytases, present naturally in the plant foods or present in yeasts or other micro-organisms used in food processing. These enzymes successively remove, one after the other, the six phosphorus groups attached to the inositol ring until it no longer binds iron and other minerals (Sandberg and Andlid, 2002; Hurrell *et al.*, 2003). The degradation of phytate is of nutritional importance because the mineral binding strength of phytate decreases and the

solubility increases when phosphate groups are removed from the inositol ring resulting in an increased bioavailability of essential dietary minerals (Sandberg and Andlid, 2002). Thus, phytases have an important role in human nutrition both for degradation of phytate during food processing and in the gastrointestinal tract.

Phytic acid has been completely degraded in weaning cereals by adding commercial exogenous phytases or by activating the native phytases by a combination of soaking, germinating, and fermentation processes (Hurrell *et al.*, 2003). Hydrolysis of phytate may occur in the gastrointestinal tract prior to the intestinal site of absorption. Because most of the essential minerals and trace elements are absorbed in the duodenal or jejunal part of the small intestine, the site and degree of phytate degradation can affect the nutritional value of a high phytate diet. Furthermore, enzymatic hydrolysis of phytate in the gastrointestinal tract leads to formation of specific isomers of inositol phosphates. If physiologically active lower inositol phosphates, formed during food processing, or degradation in the gastrointestinal tract or precursors to these are absorbed in the alimentary tract of humans which may have important health implications (Sandberg and Andlid, 2002).

Biotechnologically produced microbial phytase preparations are now commercially available and used for feed preparations (Sandberg and Andlid, 2002). In the future, their use in food processing could be feasible. Moreover, microbial genes encoding phytases with desired properties can be cloned and inserted into plants yielding increased levels of phytase and reduced phytate content (Sandberg and Andlid, 2002). In order to substantially increase Fe absorption, phytate degradation has to be virtually complete. Recent findings suggest that the inositol penta-, tetra- and triphosphates must also be degraded in order to improve mineral absorption (Sandberg, 2002).

Since phytic acid is a poor source of P for non-ruminants, the accumulation of excreted P in soils and water systems can also represent a serious environmental problem in areas of intensive animal production and insufficient purification of municipal sewage (Feil, 2001; Ogunkoya, 2006; Lorenz, 2007). In contrast to the anti-nutritional properties, dietary phytate has also been suggested to have beneficial effects, such as protection against colon cancer, arteriosclerosis and coronary heart diseases (Feil, 2001; Sandberg and Andlid, 2002; Ogunkoya, 2006; Lorenz, 2007).

Nearly all the phytic acid found in mature seeds is bound to minerals such as K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , Ba^{2+} , and Fe^{3+} to form a mixed cation salt known as phytate (Lorenz, 2007). Inorganic P and cellular P (a component of cellular membranes, DNA, RNA, etc.) are other forms of P in seeds and are generally referred to as "available P" (Lorenz, 2007). Many of the problems associated with P in sorghum grain are not due to the concentration of total P per se, but rather to the fact that most of the P is bound in phytate (Lorenz, 2007). Therefore, it would be desirable to increase the amount of available P and reduce the amount of phytate.

3.4.2. Tannins

Widely distributed polyphenols in plants are not directly involved in any metabolic process and are therefore considered secondary metabolites (FAO, 1995). Some polyphenolic compounds have a role as defense chemicals, protecting the plant from predatory attacks of herbivores, pathogenic fungi and parasitic weeds. Polyphenols in the grains also prevent grain losses from premature germination and damage due to moulds (FAO, 1995).

Certain polyphenoles are able to bind Fe, which make the complex-bound Fe unavailable for absorption (Sandberg, 2002). The amount of Fe-binding phenol galloyl groups in foods roughly

corresponds to the degree of inhibition of Fe absorption. All major types of food polyphenoles can strongly inhibit dietary non-haem iron absorption. The negative influence on Fe absorption is nutritionally the most important, especially in industrial products such as infant formulas, but more importantly in many developing countries where the diet is based on cereal and legume products (Sandberg, 2002). Cereals contain varying amounts of polyphenones and generally the amounts are considered higher in the colored seeds (Sandberg, 2002).

Phenolic compounds in sorghum can be classified as phenolic acids, flavonoids and condensed polymeric phenols known as tannins. Phenolic acids free or bound as esters are concentrated in the outer layers of the grain (FAO, 1995).

Oligomers of flavan-3-ols and flavan-3, 4-diols, called condensed tannins, occur widely in cereals and legumes (FAO, 1999). Tannin-protein complexes can cause inactivation of digestive enzymes and reduce protein digestibility by interaction of protein substrate with ionizable iron (Ogunkoya *et al.*, 2006). The presence of tannins in food can therefore lower feed efficiency, depress growth, decrease iron absorption, damage the mucosal lining of the gastrointestinal tract, alter excretion of cations, and increase excretion of proteins and essential amino acids (FAO,1999).

Some varieties of sorghum containing high tannin in the grain were found to be bird resistant (Asfaw *et al.*, 2005). Tannins are the most abundant phenolic compound in brown bird-resistant sorghum. During maturation, the brown-sorghum grain develops astringence which imparts resistance against bird and grain mould attack. This quality is important in arid and semi-arid regions where other crops fail. In some of these regions, annual losses in grain production as high as 75 percent or sometimes more have been reported (FAO, 1995).

Tannins, while conferring the agronomic advantage of bird resistance, adversely affect the grain's nutritional quality (FAO, 1995). Tannins in the grain impart an astringent taste which affects palatability, reducing food intake and consequently body growth (Ogunkoya *et al.*, 2006). Tannins bind to both exogenous and endogenous proteins including enzymes of the digestive tract, affecting the utilization of proteins (FAO, 1995). Several studies in rats, chicks and livestock have shown that high tannin in the diet adversely affects digestibility of proteins and carbohydrates and reduces growth, feeding efficiency, metabolizable energy and bioavailability of amino acids (FAO, 1995).

Enzymatic degradation of polyphenols during food processing should be a possible strategy for improvement of nutrient availability. For high-tannin cereals, it has been shown that treatment with polyphenol oxidase had a reducing effect on the phenolic content (Sandberg and Andlid, 2002). A fungal tannase was used to decompose phenolic compounds in brown beans, but the influence on mineral availability was not determined (Sandberg, 2002). Germination and fermentation of lentils were found to modify the phenolic composition (Sandberg, 2002).

The presence of tannins in sorghum not only decreases the availability of minerals per se, but also inactivates the phytase enzyme (Sandberg and Andlid, 2002). The use of lactic acid fermentation for high-tannin sorghum was therefore reported to be less effective to reduce the phytate content (Sandberg and Andlid, 2002).

3.5. Minerals

Cereals and legumes are rich in minerals but the bioavailability of these minerals is usually low due to the presence of antinutritional factors such as phytate and polyphenols (Idris *et al.*, 2005; 2007). Bioavailability is the degree to which the amount of an ingested nutrient is absorbed and

available to the body (WFP, 2006). Mineral bioavailability is affected by different factors including antinutritional factors, chemical form of the mineral, interaction with other minerals, presence or absence of certain vitamins and the type of food from which the mineral is obtained (WFP, 2006).

An adequate mineral absorption is important especially for infants, children, elderly people and people in clinical situation (Idris *et al.*, 2005). It is evident that the nutritional importance of a given food/ feed stuff depends not only on nutrient composition of raw foodstuff but also on the amount utilized (Idris *et al.*, 2007).

3.5.1. Zinc

Zinc deficiency is a public health problem, and is associated with poor growth, decreased immune function, increased susceptibility to and severity of infections, adverse outcomes of pregnancy, and neurobehavioral abnormalities (Sandberg 2002; Melaku *et al.*, 2005). Zinc is an essential trace mineral that is a component of over 200 enzymes and is known to be necessary for normal collagen synthesis and mineralization of bones, and is involved in vital processes such as mitosis, synthesis of DNA and protein, and gene expression and activation (Walingo, 2009).

Deficiency of Zn is highly prevalent in developing countries, but also in vulnerable groups with high requirements in industrialized countries, such as women of fertile age, infants and adolescents (Sandberg, 2002). Approximately one third of children in low-income countries are stunted (Walingo, 2009). Zinc deficiency is presumed to be the underlying cause of stunting and delayed sexual maturation. Zinc supplementation increases linear growth in stunted

children which suggests that these high rates of stunting may be due in part to zinc deficiency (Walingo, 2009).

Zinc nutritional status influences the absorption, transport and utilization of vitamin A. The enzyme that plays a major role in the oxidative conversion of retinol to retinal is zinc dependent, and may be adversely affected in zinc deficiency (Adeyeye *et al.*, 2000).

Zinc inhibitors like phytates and fiber are present in higher amounts in plant foods, especially cereals and legumes, and influence zinc absorption. Although phytates have been singled out as the most potent dietary inhibitor of zinc bioavailability, other known inhibitors include oxalate, fiber, and polyphenols such as tannins (Walingo, 2009).

Diets have been classified into high, medium and low-zinc availability based on the phytate-zinc molar ratio. Phytate-zinc molar ratio is used to estimate the likely absorption of zinc from a mixed diet (Melaku *et al.*, 2005; Walingo, 2009). Diets with a phytate-zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with phytate-zinc molar ratio between 5 and 15 have medium zinc bioavailability and those with a phytate-zinc molar ratio less than 5 have relatively good zinc bioavailability (Walingo, 2009). Phytate/zinc molar ratio play a major role in inhibiting zinc absorption such that zinc absorption is typically less than 15% in high phytate meals (Adeyeye *et al.*, 2000; Melaku *et al.*, 2005; Walingo, 2009).

3.5.2. Iron

Iron is a micronutrient that is most often deficient in developing countries, with children and women of reproductive age especially at risk of such deficiencies (Melaku *et al.*, 2005). Low

content and bioavailability of iron in the typical cereal-based diet is a major cause of iron deficiency (Sandberg, 2002). This is due to the high content of antinutrients in most cereals and other plant source staple foods and inadequate intake of animal foods in the diet (Sandberg, 2002; Melaku *et al.*, 2005; Malenganisho *et al.*, 2007).

In developing countries Fe deficiency, due to poor bioavailability, retards normal brain development in infants and affects the success of pregnancy by increasing premature deliveries, as well as morbidity of mother and child at or around child birth; it affects also working capacity, thus impairing socioeconomic development as well (Sandberg, 2002; Malenganisho *et al.*, 2007). Iron deficiency is the most important cause of nutritional anaemia. This arises from the low bioavailability of non-haem iron caused not only by phytate but also tannins in the diet (Melaku *et al.*, 2005).

Iron nutrition is particularly important during the weaning period, when the infant is growing rapidly and has a high demand for iron. Cereal porridges are common complementary foods during the weaning period and often provide much of the dietary iron intake because the iron contribution from human milk is low (Hurrell *et al.*, 2003). Because of the high phytate content of cereal porridges, iron absorption of native iron and fortification iron may be very low (Hurrell *et al.*, 2003; Lorenz *et al.*, 2007)). One mole of phytic acid binds 6 mol ferric irons so that even relatively small quantities of residual phytate are still strongly inhibitory (Hurrell *et al.*, 2003). Studies indicated that adding 10 mg/100 g phytic acid to bread rolls decreased iron absorption by 20% and that adding 20 mg/100 g decreased iron absorption by 40% (Hurrell *et al.*, 2003). Phytate: iron molar ratios > 0.15 are regarded as indicative of poor iron bioavailability (Melaku *et al.*, 2005). Absorption of iron from cereals can be increased by the

degradation or removal of phytic acid with a simple technology like fermentation (Hurrell *et al.*, 2003).

3.6. Fermentation

Lactic, yeast, and mixed fermentations are old methods for food processing and preservation (Jay, 2000; Holzapfel, 2002). Today, defined starter cultures and controlled conditions are frequently used. Because of the production of lactic acid and other organic acids, the pH is lowered and the phytase activity increased (Jay, 2000; Shimelis and Rakshit, 2008). Studies have shown that combined germination and lactic fermentation of white sorghum and maize gruels can yield an almost complete degradation of phytate and have antioxidant potentials (Fagbemi *et al.*, 2005; Janiszewska *et al.*, 2007). Moreover, traditional fermentation processes are increasingly attracting the attention of scientists and policy makers as a vital part of food security strategies (Abegaz *et al.*, 2002).

All over the world, fermented foods provide an important part of human diet. Fermented foods and beverages provide about 20-40% of human food supply (Fagbemi *et al.*, 2005). Traditional food fermentation is capable of improving nutrients of the food, preserve it by generating acidic condition, detoxify and reduce cooking time of the food (Jay, 2000; Fagbemi *et al.*, 2005).

Lactic acid bacteria are found to be useful in flavouring foods, in inhibiting spoilage bacteria and pathogens, in intestinal health and other health benefits related to blood cholesterol levels, immune competence and antibiotics production (Sobowale, 2007). Lactic acid fermentation is inexpensive and often little or no heat is required during the process thus making it fuel efficient (Shimelis and Rakshit, 2008).

The majority of traditional cereal-based foods consumed in Africa are processed by natural fermentation. Fermented cereals are particularly important as weaning foods for infants and as dietary staples for adults (FAO, 1999). Combining fermentation with cooking, either fermenting then cooking or cooking then fermenting improve the nutrient quality and drastically reduce the antinutritional factors to safe levels much greater than any of the other processing methods tested (Ikemefuna and Atii, 1990; Chavan *et al.*, 2006). Lactic acid fermentation of cereals generally improves extractability of minerals, probably because of the decreased content of phytic acid in the fermented cereal product (Eltayeb, 2007; Khetarpaul and Chauhan, 2007).

Over the centuries, fermentation has evolved and been refined and diversified. Today, a variety of food products are derived from this technology in households, small-scale food industries as well as in large enterprises. Furthermore, fermentation is an affordable food preservation technology and of economic importance to developing countries (Motarjemi, 2002). It enhances the nutritional quality of foods and contributes to food safety particularly under conditions where refrigeration or other foods processing facilities are not available (Motarjemi, 2002).

The increase in insoluble protein digestibility during fermentation suggests that fermentation causes structural changes in the cereal storage proteins (prolamins and glutelins), making them more accessible to pepsin attack. Thus natural fermentation, as applied in traditional African food preparation is an effective method of improving the protein digestibility of cooked cereals (Goyal and Khetarpaul, 1994; Taylor and John, 2002; Chavan *et al.*, 2006). Fermented cereal

porridges (and gels) are important staple food items for people of the West African subregion and are also important weaning foods for infants (Taiwo, 2009). Fermented foods having acidic pH are microbiologically safe and can be stored for a long time (Khetarpaul and Chauhan, 1989).

In Africa, the majority of cereal-based foods are consumed in the form of porridges and naturally fermented products (Kabeir *et al.*, 2004). Many desirable changes occur during the fermentation process of cereal grains due to the breakdown of complex compounds into simple forms and the transformation into essential constituents (Kabeir *et al.*, 2004).

The microbial groups involved in spontaneous fermentation of cereal flour include *Lactobacillus spp.*, *Acetobacter spp.* and *Saccharomyces cerevisiae* (Kabeir *et al.*, 2004; Tsaousi *et al.*, 2008). Recently, instead of the traditional lactic acid bacteria, the use of probiotics bacteria such as *Bifidobacteria* to improve the therapeutic quality of food has gained considerable interest. *Bifidobacteria* are beneficial for human beings of all ages, as they are the predominant member of the endogenous intestinal flora, capable of improving the balance of the intestinal microflora by preventing colonization of pathogens, activating the immune system and increasing protein digestion (Kabeir *et al.*, 2004).

A mean decrease of phytic acid in 64.8% after 96 hours and 39.0% after 72 hours in the fermentation of sorghum grains were indicated in a previous study (Makokha *et al.*, 2002). The extent of decrease of phytic acid differed among the grain varieties (Makokha *et al.*, 2002).

Organic acids produced, such as acetic, lactic, citric, formic and butyric acids, during fermentation potentiate zinc absorption by forming ligands with zinc (Sandberg and Andlid, 2002). Microbial fermentation enhances zinc bioavailability through hydrolysis induced by

microbial phytase enzymes (Walingo, 2009). Reduction of phytates in the diet could also favor enhanced absorption of other minerals like calcium and iron. Fermentation of cereals reduces phytate content via the action of phytases that catalyze conversion of phytate to inorganic orthophosphate and a series of myoinositols, lower phosphoric esters of phytate (Walingo, 2009).

There are differences in optimal conditions for phytate degradation between plant species (Sandberg and Andlid, 2002). Most cereal phytases have pH optima between 4.5 and 5.6, but pH optima of some legumes are neutral or alkaline (Sandberg, 2002). To optimize the food process for increased mineral bioavailability by phytate degradation, it is essential to know optimal conditions for the phytases, responsible for phytate degradation in the process (Sandberg and Andlid, 2002).

In addition to phytate, InsP_5 has been identified as inhibitor of iron and zinc absorption in human and animal studies (Sandberg and Andlid, 2002). Recently, indications were found that InsP_4 and InsP_3 also contribute to the negative effect on iron absorption by interacting with the higher phosphorylated inositol phosphates. These findings suggest that phytate degradation of not only InsP_5 and InsP_6 , but also degradation of InsP_3 and InsP_4 are favorable for improvement of iron absorption from cereals. This is probably also true for zinc absorption as a strong negative correlation was found between zinc absorption and the sum of native InsP_3 through InsP_6 from cereal and legume meals (Sandberg and Andlid, 2002).

Hydrolysis of phytate during biological food processes and preparation such as fermentation is a result of activity of phytase enzymes, naturally synthesized by plants and many microorganisms (Sandberg, 2002). Phytases (InsP_6 -phosphohydrolases) are by definition enzymes

able to hydrolyse InsP₆ to InsP₅ and inorganic phosphate (Pi). Typically, phytases are not specific for InsP₆; leading to further hydrolysis to *myo*-inositol via intermediate *myo*-inositol phosphates (penta- to monophosphates). Phytases constitute a subgroup of the family of acid phosphatases. Those that exhibit the ability to hydrolyse InsP₆ can be considered to be phytases (Sandberg and Andlid, 2002).

With respect to the position for initial hydrolysis, two types of phytases, 3- and 6-phytases, have been recognized as starting the dephosphorylation of InsP₆ at position D-3 or L-6 of the inositol ring (Sandberg and Andlid, 2002). The 3-phytases (EC 3.1.3.8) have been found to dominate among microorganisms while 6-phytase (EC 3.1.3.26) (4-phytase assuming D-configuration) has been considered to be characteristic of the seeds of higher plants (Sandberg, 2002). This is, however, not a general rule. For instance, *Escherichia coli* has been shown to produce a 6-phytase and soybean a 3-phytase (FAO, 1999; Sandberg and Andlid, 2002; Walingo, 2009).

Depending on the type of food process or whether a phytase is expected to be active in the gut, different properties should be considered in the search for optimal enzymes (Sandberg and Andlid, 2002). These may include stability at low pH and at high temperature, level of glycosylation, resistance against digestive proteolytic enzymes, high specific activity, suitable pH- and temperature optima, high level of expression, easy cultivation (of phytase producing organism) and purification, extracellular phytase, non allergic, and nontoxic (Sandberg and Andlid, 2002).

It is also reported that fermentation can reduce tannin content of cereals and other foods (Fagbemi, 2005). Reduction in tannin due to processing might have been caused by the activity of polyphenol oxidase or fermented microflora on tannins (Fagbemi, 2005).

However, regardless of the aforementioned merits of fermentation there is failure of knowledge transmission about the process from generation to generation also because of attitude formation relegating this method into categories of primitive technologies (Walingo, 2009).

4. MATERIALS AND METHODS

4.1. Chemicals and reagents

All the chemicals and reagents used in all the laboratory analyses are analytical grade.

4.2. Sorghum cultivars collection

Two sorghum cultivars (*Sorghum bicolor* (L.) Moench) were used in this study; ‘76T1#23’, and ‘Gobyie’. Both cultivars were obtained from Ethiopian Seeds Enterprise (ESE), Arsi Basic Seeds Storage and Preparation Center, Asella, Ethiopia. Both varieties collected for this study were basic seeds.

Variety 76T1#23 was released in 1979 for the moisture stressed dry lands. It is white seeded with semi-compact, semi-oval and erect panicle. It has some red spots. The glume is red or brown. Its height ranges from 120 to 140 cm, matures within 90 days and can give a yield of 25-45 quintals per hectare (Asfaw *et al.*, 2005). Gobiye variety was released in 1999/2000 cropping season. The seed is white and has a semi loose erect panicle. Its height may reach 110-140cm, matures within 90-120 days and can give a yield of 14-27 quintals per hectare (Asfaw *et al.*, 2005).

4.3. Sorghum flour preparation

In all the treatments (sample preparation, laboratory determination and data analysis), the two varieties were processed separately.

4.4. Sample preparation

The grains of both sorghum cultivars were cleaned manually to remove husks, damaged grains stones, dust, light materials, glumes, stalks, undersized and immature grains and other extraneous materials. Cleaning was done by winnowing and hand sorting. The cleaned grains

of each cultivar were dried in drying oven (Memmert, Germany) at 55⁰C for about 2 hours to facilitate and make conducive the milling process. The dried sorghum seeds of both cultivars were milled into flour using Tecator Cyclotec1093 sample mill (Sweden). All of the sorghum samples were milled to pass through a 1 mm aperture size laboratory test sieve (Endecotts Ltd., London England) to obtain a fine powder. The milled samples were then packed in airtight polythene plastic bags until further analysis.

4.5. Fermentation process

Suspensions of sorghum flour in de-ionized water were prepared in plastic containers at a concentration of 1:3 dilutions (w/v). The flour slurry was allowed to ferment naturally with only the microorganisms borne on or inside the seeds (endogenous microflora on the seeds) at room temperature (20-23⁰C) for 0, 12, 24, 36 and 48h in 10 plastic containers. The fermentation water was decanted and samples were withdrawn and transferred to aluminium dishes after each fermentation time and dried in a hot air oven-drier (Memmert, Germany) at 70 °C for 36h. Dried samples were ground with a miller (IKA-Werke, M 20) to pass a 1 mm sieve and stored for analysis. All samples were analyzed for pH, titratable acidity, moisture content, ash, crude fiber, fat, crude protein, tannins, phytate, viscosity and sensory characteristics.

4.6. pH determination

The pH of the samples was determined according to the method of Pearson (1971). For the non fermented flour 10 g of the sample was added to 30 ml of distilled water and stirred for 10 min. The pH of the slurry was determined by dipping the electrode of the pH meter (Hanna Instrument- pH 301) in the mixture. The pH of the fermented samples was determined by dipping the electrode of the pH meter in the homogenate fermented mixture slurries after the end of each fermentation period. Triplicate determinations were made in all cases. The pH meter was calibrated using pH 4.0 and 7.0 buffers.

4.7. Determination of total titratable acidity

Total titratable acidity expressed as percentage of lactic acid, was determined by titrating 30 ml of the homogenate samples used for pH determination against 0.1 N NaOH. First the distilled water (1L) used for titration was titrated with 0.1 N NaOH and the volume of 0.1 N NaOH consumed by water titration was considered as a blank. The volume of 0.1 N NaOH used for titration of the sample was noted after correcting the blank and triplicate determination was made (Pearson, 1971)

Calculation:

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

Where: V=Volume of 0.1 N NaOH used for sample titration; 0.009008=Factor equivalent in which 1ml of 0.1N NaOH =0.009008g C₃H₆O₅; W=Weight in gram of sample in the mixture (Pearson, 1971).

4.8. Determination of moisture content

Empty dishes and their lids (made of porcelain) were dried using air drying oven (Mettler, Germany) for 1 hour at 100°C, transferred to the desiccator (with granular silica gel), cooled for 30 minute, and weighed. The prepared samples were mixed thoroughly and about 5.000g of fresh samples were transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 5 hrs at 100°C. Then the dishes and their contents were cooled in a desiccator to room temperature and reweighed. Triplicates of each sample were determined. The amount of water present in a sample is considered to be equal to the loss of weight after drying the sample to constant weight at a temperature near the boiling of water.

Calculation:

$$\% \text{Moisture} = \frac{M_{\text{INITIAL}} - M_{\text{DRIED}}}{M_{\text{INITIAL}}} \times 100$$

Where, M_{INITIAL} and M_{DRIED} are the mass of the sample before and after drying, respectively.

4.9. Crude protein determination

Protein ($N \times 6.25$) was determined by the Kjeldahl method. All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulfuric acid and concentrated orthophosphoric acid containing potassium sulfate as a boiling point raising agent and selenium as a catalyst. The ammonia released after alkalization with sodium hydroxide is steam distilled into boric acid and titrated with sulfuric acid.

Digestion: About 0.5000g of fresh samples (in triplicate) were taken in a Tecator tube and 6ml of acid mixture (5 parts of concentrated ortho-phosphoric acid and 100 parts of concentrated sulfuric acid) was added, mixed, thoroughly and a 3.5ml of 30% hydrogen peroxide was added step by step. As soon as the violent reaction had ceased, the tubes were shaken for a few

minutes and placed back into the rack. A 3.0000g of the catalyst mixture (ground 0.5000g of selenium metal with 100 g of potassium sulfate) was added into each tube, and allowed to stand for about 10 min before digestion. When the temperature of the digester reached 370⁰C, the tubes were lowered into the digester. The digestion was continued until a clear solution was obtained, about 1h. The tubes in the rack was transferred into the fume hood for cooling, a 15ml of demonized water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml 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 milli-liter of water before the receiver was removed.

Titration: The distilled solution was titrated with 0.1N sulfuric acid to a reddish color.

Calculation:

mg nitrogen in the sample	= V x N x 14
g nitrogen / 100 g sample	= mg of nitrogen x 100/ mg sample
Total nitrogen (%)	= [(V-V _b) x N x 14]/ W
Crude protein (%)	= total nitrogen (%) x 6.25

Where: V = volume of sulfuric acid consumed to neutralized the sample; V_b = the volume of acid consumed to neutralize the blank; N = normality of the acid; 14=Eq. wt of nitrogen; 6.25= conversion factor from total nitrogen to crude protein

4.10. Determination of crude fat content

Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40° to 60 °C) in a soxhlet extractor. The ether was evaporated from the extraction flask. The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage.

The extraction flasks were cleaned, dried in drying oven (Memmert, Germany) at 92⁰C for 1hour, cooled in desiccators (with granular silica gel) for 30 minutes, and then weighed. The bottom of the extraction thimble was covered with about 2cm layer of fat free cotton. About 2.0000 gram of fresh samples (in triplicate) were added into the extraction thimbles, and then covered with about 2cm layer of fat free cotton. The thimbles with the sample content were placed into soxhlet extraction chamber. The cooling water was switched on, and a 70 ml of petroleum ether was added to the extraction flask through the condenser. The extraction was conducted for about 4 hrs. The extraction flasks with their content were removed from the extraction chamber and placed in the drying oven at 92⁰C for about 1h, cooled to room temperature in the desiccator for about 30 minutes and re-weighed.

Calculation:

$$W = W_2 - W_1$$

$$\text{Fat g/ 100g fresh sample} = [W * 100] / W_D$$

Where: W = weight of fat; W_2 = weight of extraction flask after extraction (wt. of flask and fat); W_1 = weight of extraction flask before extraction (wt. of flask); W_D = weight of fresh sample

4.11. Determination of crude fiber content

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

Digestion: About 1.6000g (recorded as W_3) of fresh sample was placed into a 600ml beaker, 200ml of 1.25% H_2SO_4 was added, and boiled gently exactly for 30 minutes placing a watch glass over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After 30 minute boiling, 20ml of 28% KOH was added and boiled gently for a further 30 minute, with occasional stirring.

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

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

Drying and combustion: The crucible with its content was dried for 2 hours in an electric drying oven at 130⁰C and cooled for 30 min in the desiccator (with granular silica gel), and then weighed (recorded as W₁). The crucible was transferred to a muffle furnace (Gallenkamp, size 3) and incinerated for 30 min at 550⁰C. The crucible was cooled in the desiccator and weighed (recorded as W₂).

Then the fiber was calculated as a residue after subtraction of the ash.

Calculation:

$$\text{Crude fiber g/100g} = [(W_1 - W_2) * 100] / W_3$$

Where: W₁ = weight of (crucible +sample) after drying; W₂ = weight of (crucible +sample) after ashing; W₃ = weight of fresh sample

4.12. Determination of total ash content

Ash was determined by incineration of known weights of the samples in a muffle furnace at 550⁰C (Gallenkamp, size 3) until a white ash was obtained. Organic matter was burned off and the inorganic material remaining is cooled and weighed. Heating was carried out in stages, first to derive the water, then to char the product thoroughly and finally to ash at 550⁰C in a muffle furnace. The ashing dishes (made of porcelain) were placed into a muffle furnace for 30 min at 550⁰C. The dishes were removed and cooled in a desiccator (with granular silica gel) for about 30 minutes to room temperature; each dish was weighed to the nearest g. About 2.5000g of flour sample were added into each dish. The dishes were placed on a hot plate under a fume-hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550⁰C for 6 hours, and removed from the muffle and then placed in a desiccator for 1hr to cool. The ash was clean and

white in appearance. When cooled to room temperature, each dish + ash was reweighed. Weight of total ash was calculated by difference and expressed as percentage of the fresh sample.

Calculation:

$$\% \text{ Ash (wet basis)} = \frac{M_{\text{ASH}}}{M_{\text{WET}}} \times 100$$

Where M_{ASH} refers to the mass of the ash, and M_{wet} refer to the original masses of the fresh samples.

4.13. Determination of carbohydrate

Digestible carbohydrate content was determined by difference. It was determined by subtracting the total crude protein, crude fiber, ash, and fat from the total dry weight (100 g) of the sample differences.

4.14. Determination of energy

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

$$(1\text{kJ}/100\text{g}=4.18\text{kcal}/100\text{g})$$

4.15. Mineral analysis

The mineral contents were determined by the procedure of AOAC (1984). Calcium, iron, and zinc were determined using an Atomic Absorption Spectrophotometer while phosphorous was determined by calorimetric method using ammonium molybdate. After removal of organic material by dry ashing, the residue was dissolved in dilute acid. The solution was sprayed into the flame of Atomic Absorption Spectrophotometer (Varian SpectraAA-20Plus, Varian Australia Pty., Ltd., Australia) and the absorption of the metal to be analyzed was measured at a specific wavelength.

Standard solutions: The stock standard solutions of minerals (iron, zinc and calcium) were diluted with 0.3 N HCl to concentrations that fall within the working range (0, 0.6, 1.0, 1.4, 1.8, $\mu\text{g/ml}$ for zinc analysis; 1.0, 1.5, 2.5, and 3.0 $\mu\text{g/ml}$ for calcium analysis and 0, 2.0, 6.0, 10.0 12.0 $\mu\text{g/ml}$ for iron analysis). The Atomic Absorption Spectrophotometer (AAS) used for mineral determination were calibrated using standard solutions and the reagent blank solution was run with the sample.

Mineral determination: Ashes were obtained from dry ashing. The ash was wetted completely with 5ml of 6N HCl, and dried on a low temperature hot plate. A 7ml of 3N HCl was added to the dried ash and heated on the hot plate until the solution just boils. The ash solution was cooled to room temperature at open air in a hood and filtered through a filter paper (Whatman 42, 125mm) into a 50ml graduated flask. A 5ml of 3N HCl was added into each crucible dishes and heated until the solution just boil, cooled, and filtered into the flask. The crucible dishes were again washed three times with de-ionized 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 the mark (50ml) with de-ionized water. A blank was prepared by taking the same procedure as the sample.

Calculation:

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

$$\text{Mineral content (mg/kg)} = [(a-b) \times V]/W$$

Where: W= Weight (g) of samples; V= Volume (V) of extract; a = Concentration ($\mu\text{g/ml}$) of sample solution; b = Concentration ($\mu\text{g/ml}$) of blank solution.

4.16. Phosphorous determination

Phosphorous was determined by the colorimetric method using ammonium molybdate (AOAC, 1984). It was converted to phosphomolybdate, which was reduced to a blue molybdenum compound by aminonaphtholsulphonic acid to give a blue molybdenum compound. A sample solution was obtained from mineral analysis (determination of Fe, Zn and Ca). 1 ml of the clear extract was taken from the sample solution and diluted to 100 ml with deionized water in a 100 ml volumetric flask.

A 5ml (triplicates) of the sample dilution was added into test tubes. A 0.5ml of molybdate and a 0.20ml aminonaphtholsulphonic acid was added into the test tube (sample solution) and mixed thoroughly step by step. A 0.20ml aminonaphtholsulphonic acid was added into the test tube repeatedly each time until the solution becomes clear. The solution was allowed to stand for 10 minute. The absorbance (reading A) of the solution was measured at 660 nm against distilled water. Simultaneously with sample phosphorous, standard and blank analysis were carried out.

Standard and blank solutions were prepared as above but 5 ml of working standard (reading A_S) and 5 ml of deionized water (reading A_B) in place of the sample dilution were used respectively. A standard curve was made from absorbance versus concentration and the slope was used for calculation.

Calculation:

First A_B was subtracted from all other readings

$$P \text{ mg/100g} = (A - A_B) \times 50 \times 100 \times \text{Slope} \times W_F \times 10$$

Where: A = Reading of the sample solution; A_B = Reading of the blank solution; W_F = Weight of fresh sample.

4.17. Determination of phytate content

Phytate was determined by the method of Latta and Eskin (1980) and later modified by Vantraub and Lapteva (1988). About 0.1000g of fresh samples were extracted with 10ml 2.4% HCl in a mechanical shaker (Eberbach) for 1hour 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.3% of sulfosalicylic 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 absorbance of the sample solutions were measured at 500 nm using UV-VIS spectrophotometer (Beckman DU-64- spectrophotometer, USA).

A series of standard solution were prepared containing 0, 5, 10, 20 and 40 $\mu\text{g/ml}$ 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 5 seconds. The mixtures were centrifuged for 10 minutes and the absorbances of the solutions (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 (appendix II). Phytate: mineral molar ratios were calculated using the molecular weight of IP₆=660.

Calculation:

$$\text{Phytic acid in mg/100g} = (\text{absorbance-intercept}) / (\text{slope} \times \text{density} \times \text{weight of sample} \times 10)$$

4.18. Determination of phytate and non-phytate phosphorus

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

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

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

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

4.19. Condensed tannin determination

Tannin content was determined by the method of Burns (1971) as modified by Maxson and Rooney (1972). About 2.0000 gram of sorghum flour was weighed in a screw cap test tube. The

sorghum flour was extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent (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. A 40mg of D-catechin was weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. A 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution were measured at 500nm by using water to zero the spectrophotometer, and the calibration curve was constructed from the series of standard solution using SPSS-15. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation (appendix III).

Calculation:

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

$$Tannin \text{ in mg/100g} = (absorbance-intercept) / (slope \times density \times weight \text{ of sample} \times 10)$$

4.20. Viscosity determination

The Gruels were prepared by mixing flour and water in a glass beaker at a concentration of 1:12 dilutions (w/v). The mixture of water and flour were cooked at 50°C for 10 minutes and at 92°C for other 20 minutes. The Gruel was placed in a water bath maintained at 40°C (heating

temperature) and its viscosity was measured using a Brookfield Viscometer (Model DVII Rheometer V2.0 RV; Middleboro, Massachusetts, USA). The cooked gruel was poured into the viscometer beaker, cooled to 40⁰C and viscosity was measured in centipoises, cP, using spindle number 52 at a shear rate of 3 revolution per minute (RPM). Within 3 minute, the average of the maximum and minimum viscosity reading was recorded according to the speed.

4.21. Sensory evaluation

Weaning gruels made from fermented sorghum at different hours of fermentation (0, 12, 24, 36, and 48hrs) were tasted by a panel of judges. The gruels were prepared by mixing 50g of flour with 600ml of tap water at a concentration of 1:12 (w/v) dilutions and cooked for 10 minutes at 50⁰C and for further 20 minutes at 92⁰C. 5g of sugar and 10g of milk powder were added to each sample as a flavoring and fortification agent.

The prepared gruel samples were presented to 11 weaning mother panelists. The panelists were 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 the panelists was also considered during panelist selection (not suffering from colds and allergies that affect their sensitivity for the product). The panel members were seated in an open well illuminated laboratory (independent booth) and asked to rate the gruel samples based on appearance, aroma, texture, taste and over all acceptability using a 5 point hedonic scale (where 1 = dislike very much and 5 = like very much) (appendix I). The gruels were served to the panelists in white and transparent

glass cups at about 40⁰C and asked to rinse their mouth with fresh room-temperature water that was provided to them, before next serving. The containers with the samples were coded in three digits and kept far apart to avoid crowding and for independent judgment.

Statistical analysis: Determinations were made in triplicates; errors were calculated as standard errors of the mean (SEM) and one way analysis of variance (ANOVA) in SPSS 15.0 for windows evaluation version computer programme was used to analyze the results. Means were separated using Post Hoc multiple comparison tests. Significance was accepted at the 0.05 level of probability.

5. RESULTS AND DISCUSSION

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

Unfermented flour of Gobiye and 76T1#23 cultivars had a pH value of 6.30 ± 0.01 and 6.41 ± 0.01 respectively (Table 1). Fermentation gradually reduced the pH of both cultivars with time. Fermentation of the flour for 12, 24, 36 and 48h had significantly ($p < 0.05$) dropped the pH in both cultivars.

Table1: Changes in pH and TTA (as % lactic acid) of sorghum cultivars during natural fermentation

Fermentation time (h)	Parameters			
	pH		TTA (as % lactic acid)	
	Gobyie	76T1#23	Gobyie	76T1#23
0	6.30 ± 0.01^a	6.41 ± 0.01^a	0.48 ± 0.00^a	0.26 ± 0.01^a
12	6.10 ± 0.03^b	6.24 ± 0.01^b	0.55 ± 0.01^b	0.40 ± 0.01^b
24	5.5 ± 0.00^c	5.70 ± 0.02^c	0.64 ± 0.01^c	0.46 ± 0.01^c
36	4.25 ± 0.01^d	5.06 ± 0.00^d	1.97 ± 0.01^d	0.86 ± 0.01^d
48	4.08 ± 0.02^e	4.15 ± 0.01^e	2.17 ± 0.03^e	2.00 ± 0.00^e

Values are means of triplicate samples (\pm SD). Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests.

A 35.3 % and 35.2 % decrease in pH for 76T1#23 and Gobiye varieties were observed after two days of fermentation respectively (Table1), thus indicating the production of lactic acid. The pH drop was probably the result of microbial activity on sorghum flour converting some of the carbohydrates in to organic acids such as lactic acid, citric acid and acetic acids. A rapid drop in pH with a corresponding increase in titratable acidity has been reported in lactic acid fermentation of various food grains (Elyas *et al.*, 2002; Ejigui *et al.*, 2005; Abdelhaleem *et al.*,

2008; Shimelis and Rakshit, 2008). According to these authors, the production of lactic acid bacteria during fermentation has attributed to the decrease in pH. Abdelhaleem *et al.* (2008) reported that during 16h sorghum fermentation, the pH decreased with a concomitant increase in acidity as lactic acid accumulates due to microbial activity. According to the authors TTA increased while pH decreased significantly within 16h of fermentation. Ejigui *et al.* (2005) also recorded a 37 % decrease in pH after four days of maize fermentation. The results for pH and TTA obtained by these authors were similar to the results obtained in this study. The differences observed between the varieties may be attributed to differences in the amount and nature of endogenous microflora involved in the fermentation process as reported by Shimelis and Rakshit (2008).

Unfermented flour of Gobiye and 76T1#23 cultivars had a TTA value of 0.48 ± 0.02 % and 0.26 ± 0.01 % respectively (Table 1). Fermentation gradually increased the TTA of both cultivars with time. Fermentation of the flour for 12, 24 36 and 48h had significantly ($P < 0.05$) increased the TTA in both cultivars. Within 48h of fermentation about 77.9 % and 87 % increase in TTA was observed for Gobiye and 76T1#23 cultivars, respectively. The TTA increase was probably the result of the production of lactic acid bacteria during fermentation. Spontaneous fermentation of cereals is mostly lactic acid fermentation, because chemical analysis in previous studies showed that 94.5 % of the 1.1 % total acidity was lactic and that the resulting dominant bacteria were lactic acid bacteria (Ejigui *et al.*, 2005). The titratable acidity of the fermenting material increased sharply with a concomitant decrease in the pH. In this study the increment in TTA and a decrease in pH were more pronounced within 36h of fermentation.

Like in many traditionally fermented products, the drop in pH and an increase in TTA were a means for protection from many food pathogens (Shimelis and Rakshit, 2008). Organic acids produced during fermentation also can potentially enhance Fe and Zn absorption via the formation of soluble ligands (Gibson *et al.*, 2006). Especially in weaning foods, such physicochemical properties of fermented foods is highly desirable, for the fact that children are most of the time highly vulnerable for food pathogens due to their physiological conditions (Jay, 2000; Wambugu *et al.*, 2002). According to Elyas *et al.* (2002), the increased acidity and low pH as a result of fermentation enhances the keeping quality of fermented foods, by inhibiting microbial growth and also contributing to the flavor of the processed food.

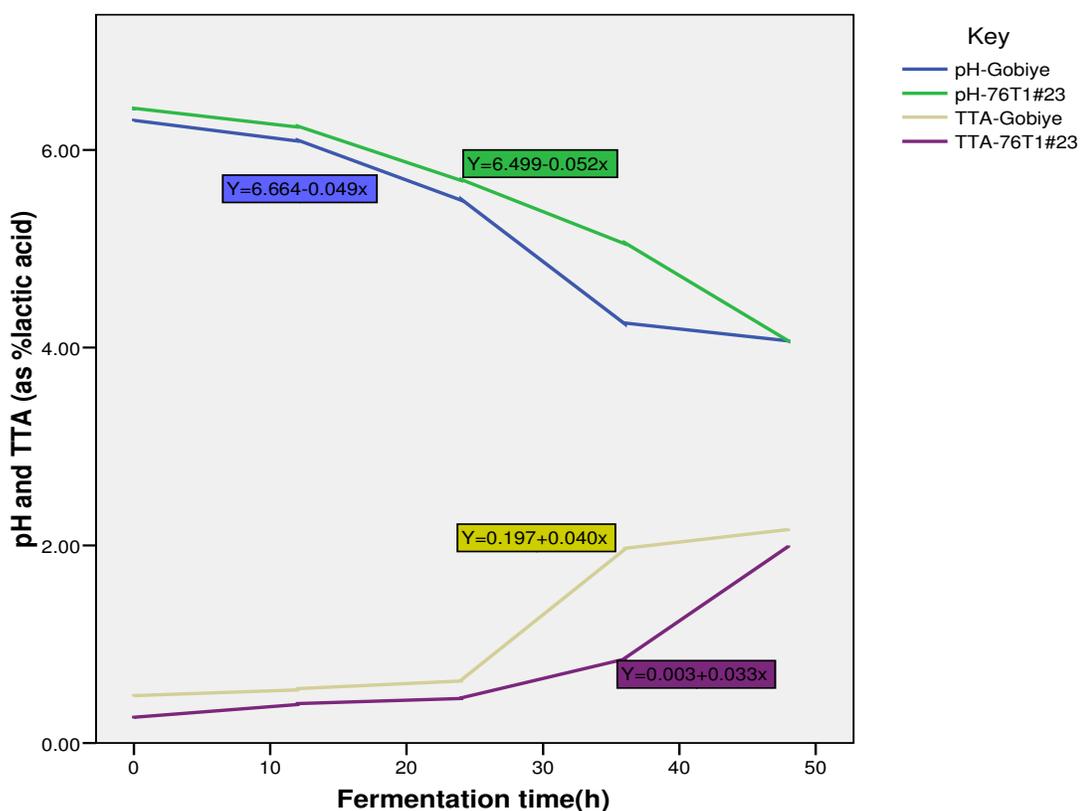


Figure 1. Changes in pH and TTA (as % lactic acid) of sorghum cultivars during natural fermentation

Figure 1 depicts changes in pH and TTA during natural fermentation. Natural fermentation showed a similar effect on pH and TTA in both cultivars. Fermentation time has a negative and positive correlation with pH and TTA, respectively. The regression model that estimates the relationship between pH and fermentation time is $Y=6.664-0.049x$ and $Y=6.499-0.052x$ for Gobiye and 76T1#23 cultivars, while the curve fit that relates fermentation time with TTA in this study is, $Y=0.197+0.040x$ and $Y=0.003+0.033x$ for the cultivars Gobiye and 76T1#23, respectively.

5.2. Effect of natural fermentation on the proximate composition of sorghum cultivars

5.2.1. Effect of natural fermentation on protein content of sorghum cultivars

The protein contents of Gobiye and 76T1#23 cultivars are shown in Tables 2 and 3, respectively. The protein content of the two genotypes increased initially as a result of fermentation. When the flour was fermented for 48h, the total protein content increased significantly ($p < 0.05$) from 10.80 ± 0.02 % to 12.56 ± 0.09 % (Table 2) for Gobiye cultivar and from 12.05 ± 0.01 % to 13.57 ± 0.05 % (Table 3) for 76T1#23 cultivar. The increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles (Elyas *et al.*, 2002). Abdelhaleem *et al.* (2008) reported that the observed increment in protein content after fermentation was probably due to shift in dry matter content through depletion during fermentation by action of the fermenting microorganisms. It may thus be apparent and not real increment. However, cells of the fermenting microorganisms could have contributed to the protein, therefore, fermentation of sorghum results in an observable increase in crude protein content. In most human diets, the protein is more limiting than others. Therefore, application of fermentation process that appears to increase the protein content even at the expense of other nutrients may be advantageous nutritionally (Abdelhaleem *et al.*, 2008).

Improvements in protein quality have also been documented after fermenting blended mixtures of plant-based complementary foods based on maize and legumes, groundnut and millet and cereal and soya bean blends (Gibson *et al.*, 2006). According to the authors such improvements may be associated with the destruction by microbial enzymes of protein inhibitors that interfere with nitrogen digestibility, or from the ability of starter cultures to synthesize certain amino acids.

Table 2: Effect of natural fermentation on the proximate composition of sorghum flour (Gobiye Cultivar).

Fermentation time(h)	M.C. (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Digestible carbohydrate (%)	Energy(Kcal/100g)
0	7.82±0.10 ^a	10.80±0.02 ^a	3.23±0.08 ^a	2.60±0.02 ^a	1.63±0.00 ^a	73.92 ^a	367.95 ^a
12	8.71±0.10 ^b	11.25±0.10 ^b	3.15±0.03 ^b	2.05±0.04 ^b	1.16±0.00 ^b	73.68 ^b	368.07 ^b
24	8.67±0.07 ^b	12.08±0.04 ^c	3.12±0.06 ^b	1.74±0.03 ^c	1.06±0.00 ^c	73.33 ^c	369.72 ^c
36	8.97±0.07 ^c	12.31±0.02 ^d	3.09±0.02 ^{bc}	1.57±0.01 ^d	0.98±0.01 ^d	73.08 ^d	369.37 ^d
48	8.95±0.00 ^c	12.56±0.09 ^c	3.05±0.03 ^c	1.55±0.02 ^d	0.96±0.02 ^d	72.93 ^e	369.41 ^d

Values are means of triplicate samples (± SD). Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests.

However, a comparison of previous studies regarding effect of fermentation on crude protein reveals variable results. Ejigui *et al.* (2005) reported a significant increase of crude protein in a 96h fermented maize flour.

Table 3: Effect of natural fermentation on the proximate composition of sorghum flour (76T1#23 Cultivar).

Fermentation time(h)	M.C. (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Digestible carbohydrate (%)	Energy(Kcal/100g)
0	7.83±0.00 ^a	12.05±0.01 ^a	3.62±0.03 ^a	2.50±0.00 ^a	1.30±0.02 ^a	72.70 ^a	371.58 ^a
12	8.70±0.31 ^b	13.09±0.03 ^b	3.27±0.02 ^b	1.98±0.01 ^b	1.13±0.02 ^b	71.83 ^b	369.11 ^b
24	8.95±0.06 ^c	13.11±0.04 ^b	3.19±0.01 ^c	1.91±0.03 ^c	1.05±0.05 ^c	71.79 ^{bc}	368.31 ^c
36	8.92±0.10 ^c	13.44±0.03 ^c	3.07±0.03 ^d	1.87±0.01 ^{cd}	0.95±0.02 ^d	71.75 ^c	368.39 ^d
48	8.94±0.04 ^c	13.57±0.02 ^d	3.05±0.08 ^d	1.82±0.05 ^d	0.94±0.07 ^d	71.68 ^d	368.45 ^d

Values are means of triplicate samples (\pm SD). Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests.

The results of this study have clearly indicated that supplementation of fermented sorghum gruels with protein sources is desirable and could be a viable and sustainable venture if undertaken on a commercial scale using appropriate technology. This is particularly so, as there is the need to improve the nutritional status of a large majority of people in the developing countries, where due to economic reasons, access to animal sources of protein is minimal (Taiwo, 2009).

5.2.2. Effect of natural fermentation on moisture and carbohydrate content of sorghum cultivars

The moisture contents of unfermented Gobiye and 76T1#23 cultivars are 7.82±0.10 % and 7.83±0.00 % respectively. During fermentation, the moisture content of both cultivars showed a

relative increment which is significant ($p < 0.05$). The maximum increment of moisture content is attained at 36 and 24h of fermentation being $8.97 \pm 0.07 \%$ and $8.95 \pm 0.06 \%$ for Gobiye and 76T1#23 cultivars, respectively. Variation in fermentation time has nothing to do with moisture content. However, the relative increment of moisture content may be attributed due to a variation in the treatment during the drying process of the fermented samples. The moisture content standard percentage recommended is 5-10 % (Olorunfemi *et al.*, 2006) while the maximum moisture content attained during the 48h of fermentation is 8.97 %. Thus fermentation has no overall effect on the standard percentage of moisture content.

The carbohydrate content of the unfermented cultivars is 73.92 % and 72.70 % for Gobiye and 76T1#23 cultivars respectively. 48h fermentation caused a significant ($p < 0.05$) reduction of total carbohydrates in both cultivars. Within this period of fermentation the carbohydrate content of Gobiye and 76T1#23 cultivars reached 72.93 % and 71.68 % respectively. The decrease in total carbohydrates calculated by difference could be due to, particularly 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).

5.2.3. Effect of natural fermentation on fat, ash and fiber contents of sorghum cultivars

Unfermented flour of Gobiye and 76T1#23 cultivars had a fat content of $3.23 \pm 0.08 \%$ (Table 2) and $3.62 \pm 0.03 \%$ (Table 3), respectively. Fermentation gradually reduced the fat content of both cultivars with time. Fermentation for 48h had significantly ($p < 0.05$) decreased the fat content to $3.05 \pm 0.03 \%$ and $3.05 \pm 0.08 \%$ for the cultivars, respectively.

Fiber and ash contents also followed similar trends. The proximate composition of the two sorghum cultivars shows that the unfermented flour samples had the highest values regarding the ash and fiber parameters examined, with an ash and fiber content of 1.63 ± 0.00 % and 2.60 ± 0.02 % for Gobiye cultivar and 1.3 ± 0.02 % and 2.50 ± 0.00 % for 76T1#23 cultivar respectively. A 48 h of fermentation has resulted in a 41.10 % and 40.40 % reduction in ash and fiber contents for Gobiye cultivar and a 27.69 % and 27.20 % drop in the ash and fiber contents for 76T1#23 cultivar respectively. The expected decrease in fiber content during fermentation could be attributed to the partial solubilisation of cellulose and hemicellulosic type of material by microbial enzymes. A previous study has reported a significant decrease of fat, ash, and fiber contents after four days of maize fermentation (Ejigui *et al.*, 2005).

The crude fiber content of infant food is expected to be low (less than 1) (Olorunfemi *et al.*, 2006) as food with high fiber content tends to cause indigestion in babies. Hence, samples with low fiber content were rated good as potential weaning foods. Fermentation as a process is promising to meet crude fiber standards in the preparation of weaning foods from locally available cereals.

The results of this study regarding the effect of natural fermentation on ash and fat contents are contradictory with the report of Goyal and Khetarpaul (1994), who stated that fat as well as ash content remained unaltered during fermentation. Khetarpaul and Chauhan (1989) also indicated that natural fermentation increased whereas pure culture fermentation decreased the fat content without affecting the ash content.

5.3. Effect of natural fermentation on phytate and tannin content of sorghum cultivars

Phytate (mg/ 100g) and tannin (mg/ 100g) contents of Gobiye and 76T1#23 cultivars of sorghum (expressed as plus or minus of the standard deviations) are shown in Table 4 as affected by different period of fermentation (0, 12, 24, 36 and 48h).

Table 4: Phytate and tannin (as D-Catechin equivalents) content of sorghum flours during natural fermentation

Fermentation time (h)	Parameters			
	Phytate (mg/100g)		Tannin (mg/100g)	
	Gobyie	76T1#23	Gobyie	76T1#23
0	432.66±2.50 ^a	405.47±0.88 ^a	34.25±0.00 ^a	80.06±0.00 ^a
12	318.58±2.50 ^b	357.17±1.09 ^b	31.70±0.24 ^b	49.92±0.53 ^b
24	284.03±1.01 ^c	339.26±1.41 ^c	28.55±0.30 ^c	42.62±0.46 ^c
36	253.57±1.59 ^d	320.32±1.46 ^d	27.08±0.71 ^d	37.40±0.32 ^d
48	130.46±0.00 ^e	305.19±1.06 ^e	17.95±0.22 ^e	33.10±0.54 ^e

Values are means of triplicate samples (\pm SD). Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests.

Phytate content of unfermented cultivars was 432.66 ± 2.50 and 405.47 ± 0.88 mg /100g for Gobiye and 76T1#23, respectively while the tannin content was 34.25 ± 0.00 and 80.06 ± 0.00 mg/ 100g for the cultivars, respectively. The statistical analysis of phytate and tannin levels of the two cultivars was also carried out and it shows that there are significant differences in the antinutrients content of the cultivars ($p < 0.05$). Differences in both phytate and tannin contents for the two cultivars could be due to both genotypic and environmental conditions.

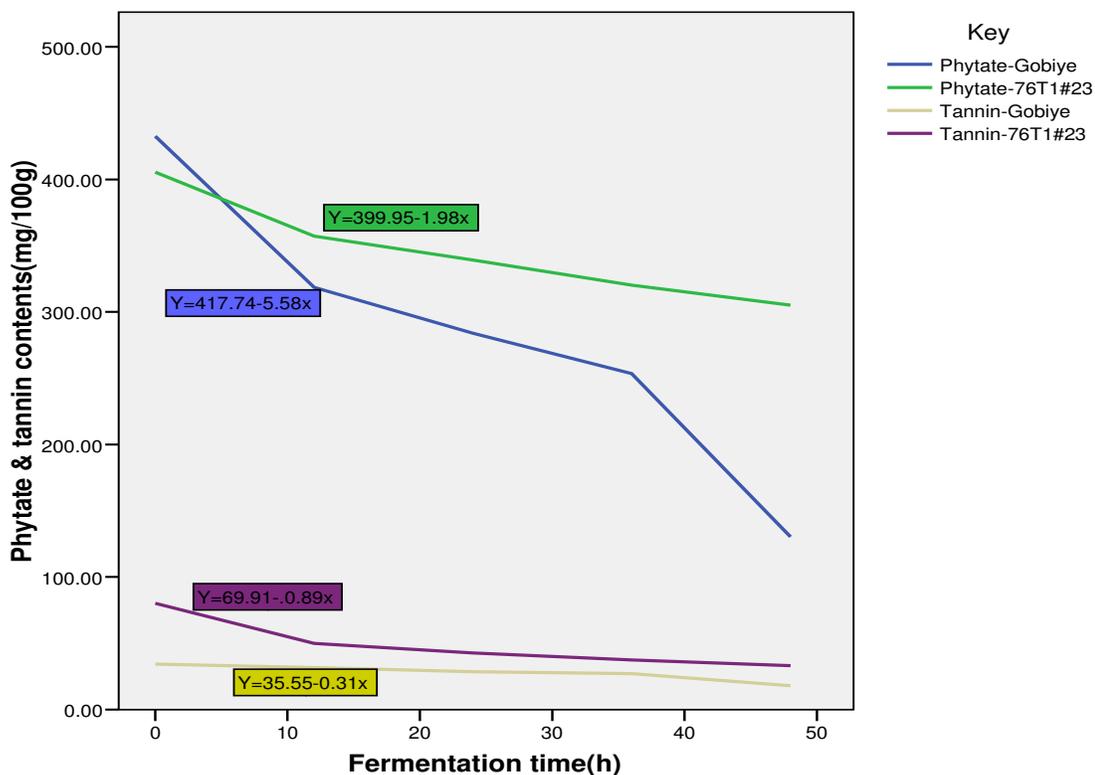


Figure 2. Effect of natural fermentation on phytate and tannin content of sorghum cultivars

Correlation analysis shows that phytate and tannin contents are negatively related with period of fermentation with Pearson correlation coefficient (r) values of 0.968 and 0.946 for phytate and tannin for Gobiye, respectively. Phytate and tannin contents of 76T1#23 cultivar also negatively correlated with fermentation time with r values of 0.967 and 0.902 for phytate and tannin, respectively.

The regression model that estimates the relationship between phytate content with respect to period of fermentation is $Y=417.74-5.58x$ and $Y=392.96-1.98x$ for Gobiye and 76T1#23 cultivars, while the curve fit that relates fermentation time with tannin content in this study is that, $Y=35.35-0.31x$ and $Y=69.91-0.89x$ for the cultivars, respectively (Figure 2).

Fermentation of the cultivars for 12h significantly ($p < 0.05$) reduced total phytate from 432.66 to 318.58 mg/100g and 405.47 to 357.17 for Gobiye and 76T1#23, respectively (Table 4). The reduction rate continued and reached its maximum value of 130.46 mg/100g and 305.19mg/100g for the cultivars, respectively when the flour was fermented for 48h. It has been suggested that the loss of phytate during fermentation could be a result of the activity of native phytase and/or the fermentative microflora by different workers (Elyas *et al.*, 2001; Abdelhaleem *et al.*, 2008; Shimelis and Rakshit, 2008). Most of the reduction of phytate occurred during the 48 hour of fermentation. This may be due to the prevailing pH which is considered to be an optimum pH for microbial phytase activity, since all enzymes have a specific pH in which they function most proficiently. It has been stated that the phytate is insoluble at pH 6; the microbial phytase activity is inhibited below pH 5.0 (Abdelhaleem *et al.*, 2008).

According to Sandberg and Andlid (2002) there are differences in optimal conditions for phytate degradation between plant species. However, most cereal phytases have pH optima between 4.5 and 5.6, while pH optima of some legumes are neutral or alkaline (Sandberg, 2002). Hurrell *et al.* (2003) indicated 5-5.5 is an optimal pH range of phytase activity. Other workers also reported that 4-6 as pH optima of phytase activity (Shimelis and Rakshit, 2008).

In this study, it was found that after 48h fermentation, the percentage decrease in phytate content was 69.85 and 24.73 for Gobiye and 76T1#23 cultivars, respectively. The results of this study are in agreement with those reported by Abdelhaleem *et al.* (2008) and Makokha (2002), who stated that fermentation of sorghum, produces significant loss in phytate. Fagbemi *et al.*

(2005) also concluded that fermentation is the most effective processing technique that reduced phytic acid in the cereal flours.

However, the percentage reductions in phytate achieved within 48h by the two cultivars are significantly different ($p < 0.05$). In 48h fermentation more phytate reduction was achieved in Gobiye (69.85 %) than 76T1#23 (24.73 %). This may be ascribed due to the higher tannin content of 76T1#23 cultivar as compared to Gobiye, since high tannin content acts as inhibitor of phytase activity (Hurrell *et al.*, 2003). The presence of tannins in sorghum not only decreases the availability of nutrients per se, but also inactivates the phytase enzyme. The use of lactic acid fermentation for high-tannin sorghum was therefore reported to be less effective to reduce the phytate content (Sandberg and Andlid, 2002).

Fermentation of the cultivars for 12h significantly ($p < 0.05$) reduced total tannin, expressed as catechin equivalents (CE), from 34.25 to 31.70 mg /100g and 80.08 to 49.92 for Gobiye and 76T1#23, respectively (Table 4). The reduction continued and reached its maximum value of 17.95mg /100g and 33.10 for the cultivars, respectively when the flour was fermented for 48h. The reduction in tannin content of fermented samples is significant ($p < 0.05$) compared to unfermented samples in all fermentation times. The effect of fermentation of Gobiye on tannins gave results similar to those obtained for 76T1#23 cultivar. Reduction in tannin contents due to fermentation might have been caused by the activity of polyphenoloxidase or tanniase of fermenting microflora on tannins (Fagbemi *et al.*, 2005).

For both cultivars the results revealed that fermentation greatly enhances the removal of the antinutritional factors which are believed to be responsible for unavailability of both proteins and divalent minerals (Abdelhaleem *et al.*, 2008).

5.4. Effect of natural fermentation on mineral contents of sorghum cultivars

The mineral contents of the two sorghum cultivars (Gobiye and 76T1#23) were shown in Table 5. The values of the unfermented flour in both cultivars were different slightly from some of the fermented samples and significantly ($p < 0.05$) from others in all the parameters examined without maintaining uniformities along with period of fermentation time. The total mineral values of both naturally fermented cultivars were lower as compared to the unfermented samples of the cultivars. The contents of calcium, iron, zinc and phosphorus in unfermented sorghum cultivars were 15.67, 7.8, 2.06 and 224. for Gobiye and 16.18, 8.26, 2.09 and 214.42 for 76T1#23 in mg/100g respectively. 48h fermented Gobiye had 15.57 mg of calcium, 7.59mg iron, 1.96 mg of zinc and 16 223.97 mg of phosphorus per 100 g of sample. The corresponding values of 76T1#23 cultivar after 48h of fermentation is that 16.01mg of calcium, 8.13mg of iron, 1.96mg of zinc and 214.25 mg of phosphorus per 100 g of sample. A significant ($p < 0.05$) variation was noticed in the total amount of calcium, iron, zinc and phosphorus in both cultivars within 48h of fermentation.

The results of the present study contradicts with the observation made by Ejigui *et al.* (2005) that fermentation does not have an overall effect on the contents of total minerals. On the other hand the reduction of total minerals in some of the samples after fermentation may be

Table 5: Effect of natural fermentation on the mineral content of flour of sorghum cultivars

Fermentation time(h)	Parameters							
	Ca (mg/100g)		Fe (mg/100g)		Zn (mg/100g)		P (mg/100g)	
	Gobyie	76T1#23	Gobyie	76T1#23	Gobyie	76T1#23	Gobyie	76T1#23
0	15.67±0.02 ^a	16.18±0.04 ^a	7.8±0.06 ^a	8.26±0.02 ^a	2.06±0.03 ^a	2.09±0.01 ^a	224.16±0.02 ^a	214.42±0.04 ^a
12	15.69±0.05 ^a	16.10±0.00 ^b	7.68±0.01 ^b	8.21±0.07 ^{ab}	1.97±0.00 ^b	1.96±0.06 ^b	224.12±0.01 ^a	214.37±0.00 ^a
24	15.64±0.00 ^{ab}	16.07±0.01 ^{bc}	7.75±0.0 ^a	8.18±0.03 ^b	2.00±0.05 ^{ab}	1.94±0.04 ^{bc}	224.12±0.03 ^a	214.31±0.03 ^b
36	15.60±0.03 ^b	16.05±0.00 ^{bc}	7.60±0.05 ^c	8.16±0.01 ^b	1.98±0.01 ^b	2.01±0.01 ^{bd}	224.01±0.05 ^b	214.29±0.07 ^b
48	15.57±0.01 ^{bc}	16.01±0.02 ^c	7.59±0.01 ^c	8.13±0.04 ^{bc}	1.96±0.02 ^b	1.96±0.01 ^b	223.97±0.02 ^b	214.25±0.02 ^b

Values are means of triplicate samples (\pm SD). Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests.

ascribed due to the microorganisms could have utilized some of the hydrolyzed elements for their metabolic activities and lost through decantation and /or the minerals could have been lost in the fermentation medium and decant of fermentation water during the drying process.

The phytate: calcium molar ratio was above the critical molar ratio of 0.24 (Frontela *et al.*, 2009), in all the fermented and unfermented samples of sorghum cultivars. Thus the result indicates unfavorable Ca absorption due to the influence of phytate. High calcium levels in foods can also promote the phytate-induced decrease in zinc bioavailability when the [calcium x phytate]: [zinc] millimolar ratio exceeds 0.5 (Kelbessa and Narasimha, 1998; Adeyeye *et al.*, 2000; Melaku *et al.*, 2005). However, in this study values > 0.5 were not observed in all the samples of both sorghum cultivars which indicate that the samples are poor in calcium content.

Thus, the possible contribution of calcium in such type of sorghum based weaning food would not exacerbate the low bioavailability of zinc.

Table 6: Effect of natural fermentation on molar ratios of phytate: zinc, phytate: calcium [Calcium x Phytate]: [Zinc] and proportion of phosphorus as phytate in sorghum cultivars

Fermentation time(h)	Parameters							
	Phytate: Ca		Phytate:Fe		Phytate:Zn		[Calcium x phytate] / [zinc] (mol/kg)	
	Gobyie	76T1#23	Gobyie	76T1#23	Gobyie	76T1#23	Gobyie	76T1#23
0	1.67 ^a	1.52 ^a	4.69 ^a	4.15 ^a	20.82 ^a	19.23 ^a	0.08 ^a	0.08 ^a
12	1.23 ^b	1.34 ^b	3.51 ^b	3.68 ^b	16.03 ^b	18.06 ^b	0.06 ^a	0.07 ^a
24	1.10 ^c	1.28 ^b	3.10 ^c	3.51 ^c	14.07 ^c	17.33 ^c	0.06 ^a	0.07 ^a
36	0.99 ^d	1.21 ^c	2.82 ^d	3.32 ^d	12.69 ^d	15.79 ^d	0.05 ^a	0.06 ^a
48	0.51 ^e	1.16 ^c	1.45 ^e	3.18 ^e	6.60 ^e	15.43 ^e	0.03 ^a	0.06 ^a

Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests.

The bioavailability of iron was low because of the high levels of phytic acid. This resulted in phytate: iron molar ratios > 0.15 which is regarded as indicative of poor iron bioavailability (Melaku *et al.*, 2005) in all the samples of sorghum cultivars examined.

Phytate: zinc molar ratios > 15 , indicative of poor zinc bioavailability (Adeyeye *et al.*, 2000; Melaku *et al.*, 2005; Walingo, 2009), were found in all the samples analyzed, except for 24, 36 and 48h fermented flours of Gobyie cultivar which have a phytate: Zinc molar ratio of 14.07, 12.69 and 6.60, respectively (Table 6).

Children in rural Ethiopia are especially very prone to deficiencies of minerals and trace elements, as they eat from the family dish and often cannot meet their specific nutrient needs. This is supported by Melaku *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 (Kelbessa and Narasimha, 1998; Adeyeye *et al.*, 2000).

In general, phytate: mineral ratio was decreased significantly after fermentation for all the parameters examined, even if the critical values were not achieved in most samples. The lower phytate: mineral ratios from the fermented sorghum cultivars 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) (Figure 3). Thus fermentation enhances bioavailability of minerals by degrading phytate with microbial and native phytases that entangle macro- and trace- elements. This study is in accord with those of fermented rice-dehulled blackgram blends (Khetarpaul and Sharma, 1997) and fermented cereal based complementary foods (Kelbessa and Narasimha, 1998 ; Odumodu, 2007). These studies indicated that fermentation hydrolyzed antinutrients from their organic bonds to increase mineral bioavailability.

The decrease in the phytate: Zinc molar ratio during 24-48h fermentation of Gobiye cultivar to realize bioavailability of zinc could be attributed to ability of phytase to hydrolyze more phytates due to the reduction of pH that favors phytase activity without the relative interference of tannin.

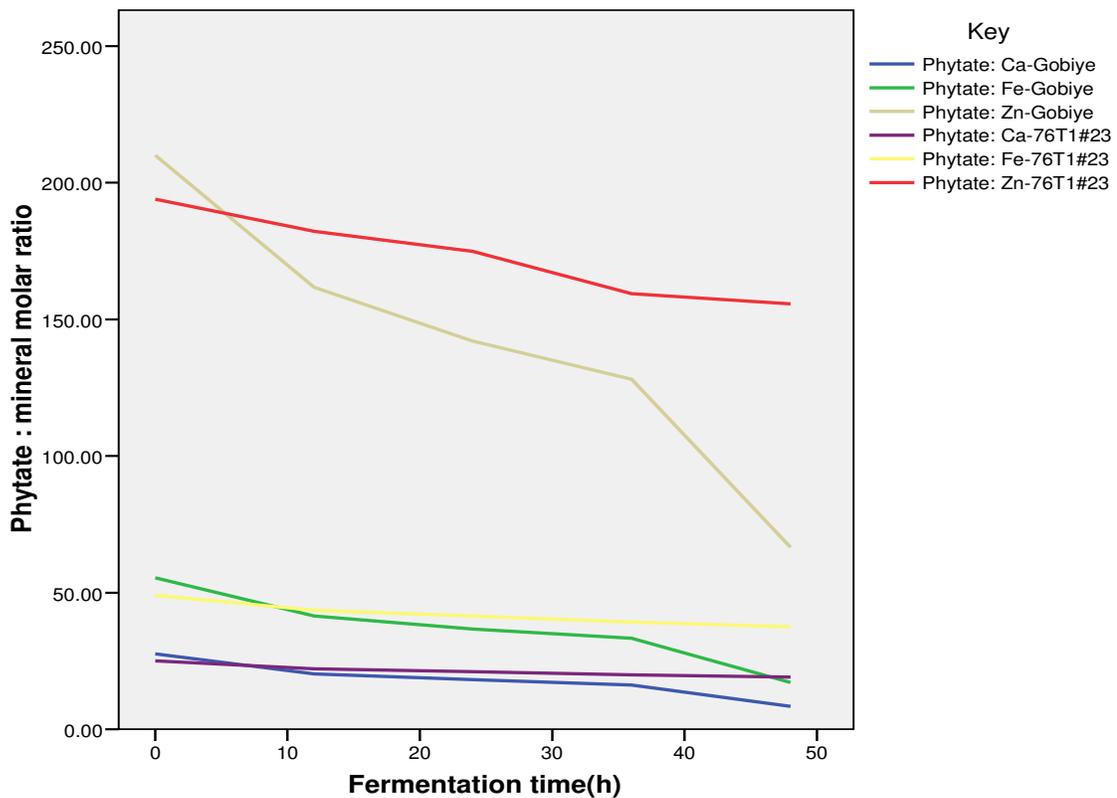


Figure 3. Effect of natural fermentation on phytate: mineral molar ratio of sorghum cultivars

There are several conflicting reports about mineral values in lactic acid fermentation. Abdel-Rahaman *et al.* (2008) observed an increase in both total and available amounts of calcium, iron, zinc and phosphorous after 14h of pearl millet lactic acid fermentation. According to Odumodu (2007), fermentation was found to enhance both the macro elements and the micronutrients of the fermented grains up to 72 h, thereafter there were fluctuations in values which could be attributed to the metabolic activities of the microorganisms. However, all settled that fermentation ameliorates bioavailability of minerals in one way or another.

5.5. Effect of natural fermentation on phytate phosphorous and non phytate phosphorous contents of sorghum cultivars

Phosphorous analysis showed values of 224.16 and 214.42 mg per 100g of sample for Gobiye and 76T1#23 cultivars, respectively. The percentage of phytate P /total was 54.39 and 53.29 for Gobiye and 76T1#23 cultivars, respectively. Results obtained in this study shown a strong positive linear correlation between phytic acid and total phosphorous with a correlation coefficient of 1.000.

Abdel-Rhaman *et al.* (2005) concluded that, in various seeds, phytic acid positively correlates with total P, correlation coefficients being greater than 0.90. Factors that affect the total phosphorous content, such as soil, available phosphorous, variety, climatic condition and fertilizers, can influence the phytic acid concentration (Muzquiz *et al.*, 1999).

Fermentation lowered the levels of phytate P in all the samples with a simultaneous increase in non phytate phosphorous significantly ($p < 0.05$) (Table 7). Within 48h of fermentation the non phytate phosphorus increased by 45.39 % for Gobiye and by 21.90 % for 76T1#23 cultivars, respectively. Thus, the hydrolytic reduction of phytic acid during fermentation may have contributed the bioavailability of phosphorus. Correlation coefficients shown a significant negative correlation between phytate and non phytate phosphorus. Hence, the lower the phytate phosphorus, the more bioavailable was phosphorus in the fermented samples. Generally, diets with phosphorus as phytate (%) ≤ 60 % are regarded as being adequate in bioavailable phosphate (Melaku *et al.*, 2005). Thus the effect of phytate to lay on the line of phosphorous bioavailability was not observed in this study. However, the high proportion of phosphate as

phytate has consequences for bioavailability of minerals and trace elements (Melaku *et al.*, 2005).

Table7: Effect of natural fermentation on phytate phosphorous and non phytate phosphorous contents of sorghum cultivars

Fermentation time(h)	Parameters							
	P (mg/100g)		Phytate P (mg/100g)		Non Phytate P (mg/100g)		Proportion of phosphorus as Phytate (%)	
	Gobyie	76T1#23	Gobyie	76T1#23	Gobyie	76T1#23	Gobyie	76T1#23
0	224.16 ^a	214.42 ^a	121.92 ^a	114.26 ^a	102.24 ^a	100.16 ^a	54.39 ^a	53.29 ^a
12	224.12 ^a	214.37 ^a	89.78 ^b	100.65 ^b	134.34 ^b	113.72 ^b	40.06 ^b	46.95 ^b
24	224.12 ^a	214.31 ^b	80.04 ^c	95.60 ^c	144.08 ^c	118.71 ^c	35.71 ^c	44.61 ^c
36	224.01 ^b	214.29 ^b	71.46 ^d	90.27 ^d	152.55 ^d	124.02 ^d	31.90 ^d	42.13 ^d
48	223.97 ^b	214.25 ^b	36.76 ^e	86.00 ^e	187.21 ^e	128.25 ^e	16.41 ^e	40.14 ^e

Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests.

The reduction in phytate phosphorus during fermentation may be due to the phytate hydrolysis by phytase elaborated by fermenting microflora (Sandberg and Andlid, 2002; Hurrell *et al.*, 2003). Cleavage of phosphorus from phytic acid may explain the improved availability of phosphorus in fermented sorghum cultivars. Natural fermentation has been reported earlier to increase the HCl-extractability of phosphorus with a corresponding decrease in the phytic acid content of pearl millet flour (Khetarpaul and Sharma, 1997). A corresponding decrease in phytate phosphorus and enhancement in the non phytate phosphorus were noticed in the present study. Therefore, consumption of fermented sorghum gruel may help to ameliorate prevalent

mineral deficiencies caused by their limited bioavailability from the unfermented ones and may lead to better mineral status in children from developing countries (Khetarpaul and Sharma, 1997).

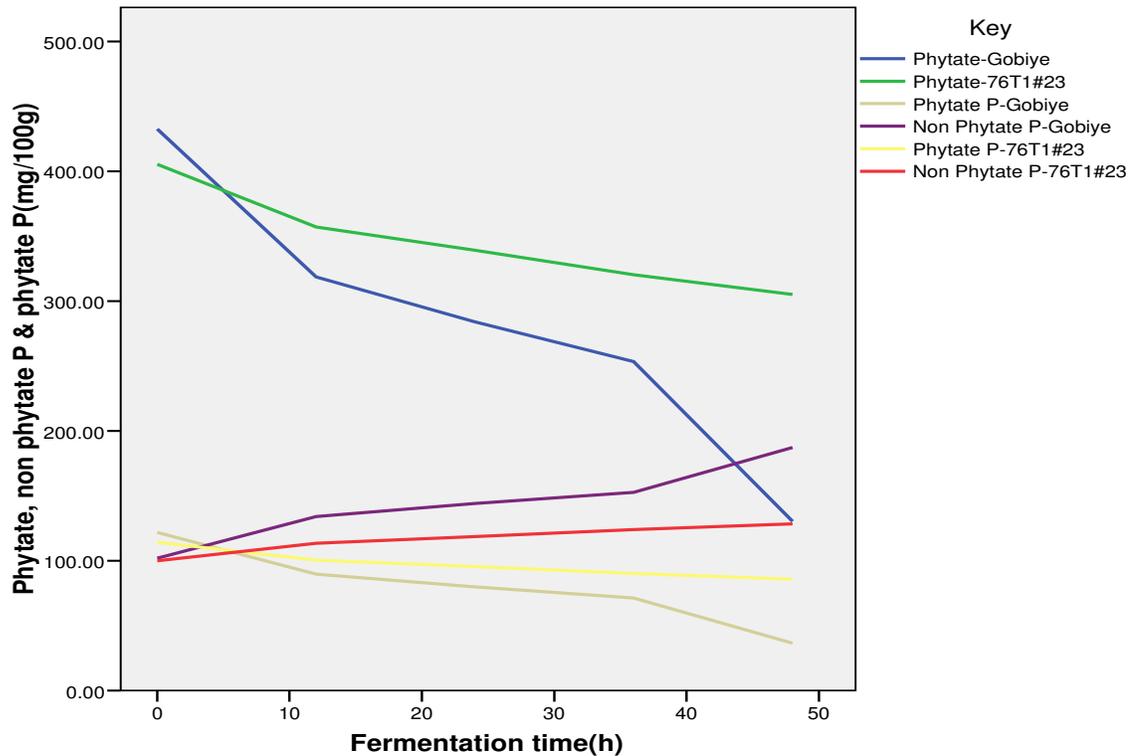


Figure 4. Effect of natural fermentation on phytate phosphorous and non phytate phosphorous contents of sorghum cultivars

5.6. Effect of natural fermentation on viscosity of sorghum cultivars

Unfermented Gobiye and 76T1#23 cultivars (8.33% solids) have a viscosity of 5292 and 5576 cP, respectively. The viscosity decreased significantly ($p < 0.05$) with increased period of fermentation in all samples and reached 3107 and 3149 cP within 48h of fermentation for Gobiye and 76T1#23 cultivars, respectively. Previous studies have reported that a pH below 5.5 decreased the viscosity of the paste in cereal starch gruels (Guyot *et al.*, 2007). The decrease in

viscosity during fermentation may be ascribed due to increased α -Amylase activity by taking the advantage of pH reduced medium.

Table 8: Effect of natural fermentation on viscosity of sorghum cultivars

Fermentation time(h)	Viscosity(cP)	
	Gobiye	76T1#23
0	5292 ^a	5576 ^a
12	4261 ^b	4584 ^b
24	3438 ^c	3583 ^c
36	3298 ^d	3364 ^d
48	3107 ^e	3149 ^e

Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests.

This enzyme can hydrolyze amylose and amylopectin to dextrans and maltose, thus reducing the viscosity of thick cereal porridges (Gibson *et al.*, 2006).

5.7. Effect of natural fermentation on the sensory characteristics of sorghum gruel

Table 9 shows the results of the sensory evaluation carried out on gruels prepared from sorghum flours (Gobiye cultivar) fermented for different length of time. The preference of the panelists for the characteristic taste and overall acceptability of the fermented gruel decreased with the increasing period of fermentation. The result also shown that the unfermented and 12h fermented gruel samples were rated significantly ($p < 0.05$) higher in terms of appearance and

aroma compared to other samples (24, 36 and 48h fermented). The length of fermentation therefore affected the perceived characteristic of appearance, aroma and taste and overall acceptability of the products.

The microbial activities and organic acid production which increased as the fermentation progressed may account for the perceived changes in the taste, aroma and overall acceptability of the gruels fermented for different length of time. A previous study on fermented cassava indicated that the microbial population increased with increase in period of fermentation contributes significantly to the odor and overall acceptability of the product (Oyewole and Ogundele, 2001). There was no significant difference ($p < 0.05$) in the appearance and aroma of the unfermented and 12h fermentation for gruel samples. The panelists however, noted that appearance, aroma, texture, taste and overall acceptability of the gruel prepared from sorghum flour that was subjected to 48h fermentation differ from others and least acceptable.

Table 9: Effect of natural fermentation on the sensory characteristics of sorghum gruel

Sensory Attributes					
Fermentation time (h)	Appearance	Aroma	Texture	Taste	Over all acceptability
0	4.36±0.51 ^a	4.36±0.83 ^{ab}	3.36±1.12 ^a	4.27±0.78 ^a	4.27±0.46 ^a
12	4.45 ±0.52 ^{ab}	4.45 ±1.04 ^a	3.09 ±0.94 ^b	4.09 ±0.83 ^b	4.09 ±0.83 ^b
24	4.09± 0.70 ^{ac}	3.91 ±0.83 ^b	3.27 ±1.34 ^c	3.73 ±1.19 ^c	3.91±0.83 ^c
36	3.82 ± 0.98 ^d	3.00 ± 0.89 ^{bc}	3.36 ± 1.28 ^a	3.64 ± 1.02 ^d	3.64 ± 0.81 ^d
48	3.91± 0.54 ^d	2.82 ± 1.40 ^c	2.82 ± 0.98 ^d	2.45±1.12 ^c	2.91 ± 1.04 ^e

Values are means of 11 observations; any two means followed by the same superscript letter for the same attribute are not significantly different at 5% level of significance as assessed by Post Hoc multiple comparison tests.

For the attribute of texture preference, there was no uniform significant difference ($p < 0.05$) between the gruel samples prepared from 0 to 48h fermented sorghum flours along with period of fermentation.

This study indicated that fermentation has effect on some sensory characteristics of fermented sorghum gruel. Thus fermentation period of 12 to 24 hours is recommended for the production of good quality and sensory characteristics of weaning gruel prepared from fermented sorghum flour. Weaning mothers should be encouraged to prepare 12 to 24h fermented sorghum based weaning gruels that may blend with either legumes or milk powder by adding some amount of sugar. The milk powder or legume would supply the required proteins and other nutrients while sugar enhances taste and flavor. Such food items may be relatively available and affordable to the low- and middle-economic mothers while still meeting the nutritional requirements of weaning babies at no exaggerated extra cost.

6. CONCLUSION AND RECOMMENDATION

Infant malnutrition and deficiency of micronutrients are highly prevalent and even increasing in parts of several developing countries. Factors of immediate and direct influence to these nutritional disorders are inadequate food consumption and diseases, which usually interact in a mutually reinforcing manner. In addition, the bioavailability of many nutrients in cereal diets is usually low, and this will significantly contribute to the nutritional inadequacy. To improve the nutrient intake, food preparation technologies have been advocated that will effectively increase the nutrient availability of cereal diets. These technologies must, however, be simple, easily understood and culturally acceptable, and the food products must be affordable, in terms of economy and labour input.

Utilization of sorghum fermentation to lower phytic acid and tannin contents and to improve the extractability of major and trace minerals is a promising and simple method. The rate of reduction of phytate and tannin contents with a concomitant increment of minerals availability depends on the length of fermentation time.

Natural fermentation of sorghum is a more acceptable process as it is inexpensive, fuel efficient method and environmentally friendly by which people can obtain good quality food and this process can only be performed at their own homes. Furthermore, it does not require the use of pure cultures, which might be costly even if they are available. It is also possible that by repeated usage of the naturally occurring endogenous microorganisms for fermentation, a more potent microbial flora capable of breaking down antinutrients and increasing mineral bioavailability will accumulate naturally.

However, regardless of the aforementioned merits of fermentation there is insufficient knowledge transfer of this traditional technology from generation to generation because of attitude formation relegating this technology into categories of primitive technologies.

In order to enhance the potentials of natural fermentation, there is need for further research on its preservation, technology transfer, technology improvement and its socio-economic implications. Natural fermentation processes will be greatly improved with the development and application of quality and safety systems and with community awareness intervention programmes.

Further research, especially in developing countries, should be directed towards the search for other strains of microorganisms that can reduce/eliminate antinutritional and toxic components in foods during fermentation to increase their utilization and application at the industry level. Depending on the type of food being processed, different properties of enzymes should be also considered in the search for antinutrient hydrolysis. These may include stability at low pH and at high temperature, level of glycosylation, resistance against digestive proteolytic enzymes, high specific activity, suitable pH and temperature optima, high level of expression, easy cultivation (of phytase producing organism) and purification, extracellular phytase, non allergic, and nontoxic. Researchers should also take into consideration the incorporation of phytase enzymes into cereal plants to enhance the nutritional value in addition to yield improvement by the application of biotechnology.

This study indicated that fermentation has effect on some sensory characteristics of fermented sorghum gruel. Thus a fermentation period of 12 to 24 hours is recommended for the production of good quality and sensory characteristics of weaning gruel prepared from

fermented sorghum flour. Weaning mothers should be encouraged to prepare 12 to 24h fermented sorghum based weaning gruels that may blend with either legumes or milk powder by adding some amount of sugar. The milk powder or legume would supply the required proteins and other nutrients while sugar enhances taste and flavor. Such food items may relatively be available and affordable to low- and middle-economic mothers while still meeting the nutritional requirements of weaning babies at no exaggerated extra cost. Weaning food industries should also be encouraged bring into focus the introduction of fermented weaning foods in Ethiopia.

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APPENDICES

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

Panelist Code: -----Date: -----

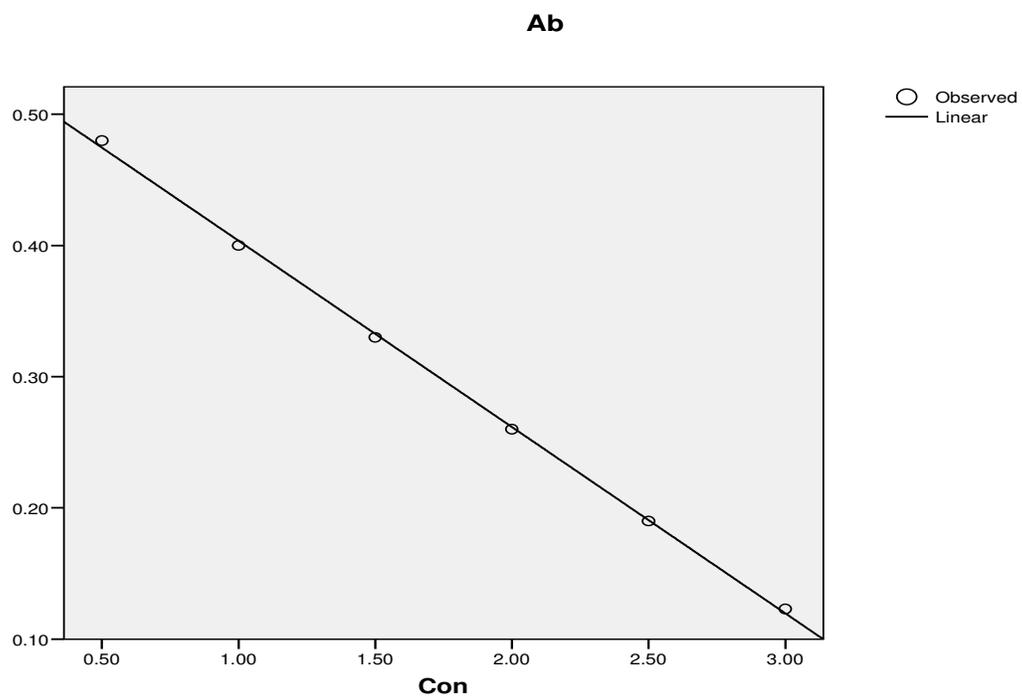
Please evaluate the gruel sample you have provided and indicate how much you like or dislike it for appearance, aroma, taste, texture and overall acceptance by a right score. Rinse your mouth with water after you evaluate each sample and before you start the next one.

Sample Code: -----

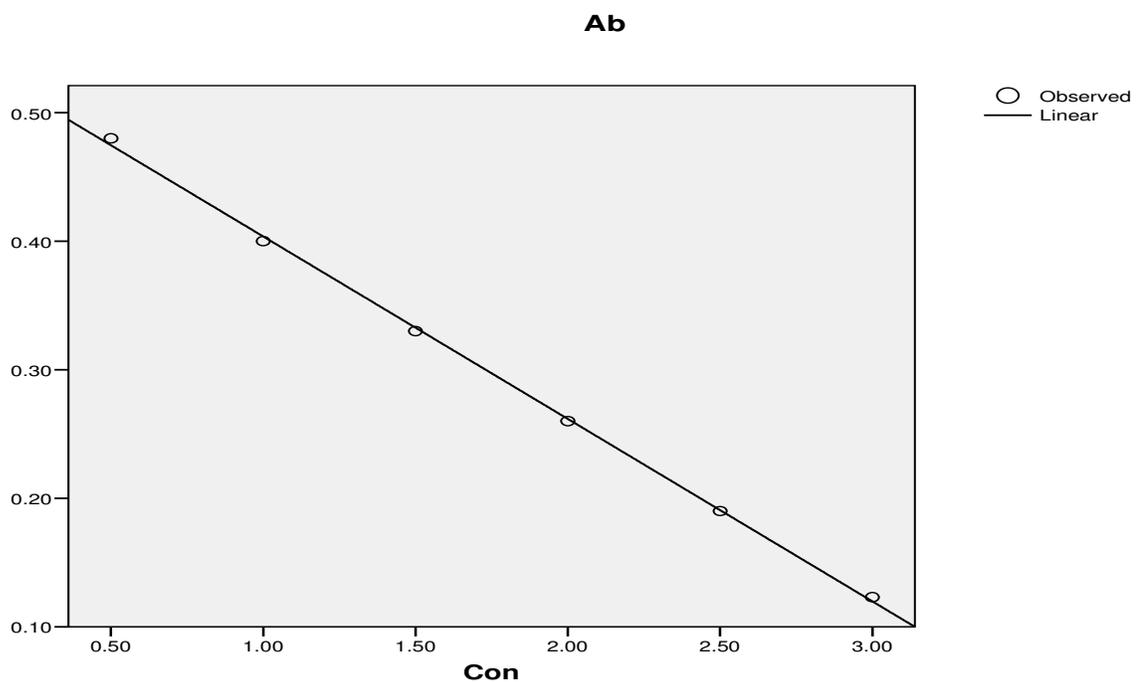
Degree of liking or disliking	Sensory Characteristic Attributes				
	Appearance	Aroma	Taste	Texture	Overall Acceptance
5.Like very much					
4.Like slightly					
3.Neither like nor dislike					
2.Dislike slightly					
1.Dislike very much					

Comments:-----

APPENDIX II: A STANDARD CURVE FOR PHYTATE DETERMINATION ($R^2=0.999$)



APPENDIX III: A STANDARD CURVE FOR TANNIN DETERMINATION ($R^2=0.998$)



DECLARATION

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

Name: Mihiret Kassa: _____

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

Mr. Kelbesa Urga: _____

Prof. Negussie Retta _____

Place and date of submission: School of Graduate Studies, Addis Ababa University, Addis Ababa, Ethiopia. June, 2009.