ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

CENTER FOR FOOD SCIENCE AND NUTRITION

Effects of fermentation on Nutritional, Anti-nutritional, Phytochemical and Functional properties of Wild oat (*Avena abyssinica*)

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College of Natural and Computational Sciences

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Declaration

I, the undersigned, declare that, this is original work and has never been presented in any other University as well as research institutes and all the source materials used for writing the thesis have been fully acknowledged.

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This thesis has been submitted for examination with my approval as University advisor.

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List of abbreviations

AACC	American Association of Cereal Chemistry
AAS	Atomic Absorption Spectrophotometer
AOAC	Association of Official Analytical Chemists
AVAs	Avenanthramides
DF	Dietary Fibers
EC	European Commission
FDA	Food and Drug Administration
GBIF	Global Biodiversity Information Facility
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
NPN	Non-protein Nitrogen
RDS	Rapidly Digestible Starch
RS	Resistant Starch
SDS	Slowly Digestible Starch
WAC	Water Absorption Capacity

Abstract

Oats or Avena comprises about 30 species; one of the examples A.sativa and A.abyssinica.In Ethiopia A.abyssinica is made into 'injera' and other products. Underutilized crops are those which are neither cultivated in an organized forming system nor processed by established commercial processing. In this research, effect of natural fermentation with time on proximate composition physicochemical, phytochemical, anti-nutritional, functional and acute toxicity of A.abyssinica flour was studied. A. abyssinica sample was collected from north Show zone, Angolela and Tera woreda Chacha area. A total of 15kg sample was collected from a local market, cleaned, dehulled, and finally packed in polyethelene bags. All the analysis was performed triplicate. results were expressed as mean ± standard error (SE). In the proximate analysis; crude protein, carbohydrate, moisture, crude fat, and Fiber content ranged from 11.46 to 12.83, 72.68, 8.33 to 11.38, 7.00 to 8.00% and 7.18 to 9.92% respectively. From the current study, Phytochemical properties such as total phenolic and flavonoid contents was ranged from 374.7 to 652.8 mg GAE/g and 161.42 to 184 mgDE/g respectively. Physicochemical property revealed that, hundred seed weight of A. abyssinica seeed was 3.11g also PH ranged from 0.02 to 0.2 respectively. In the determination of functional properties six parameters were studied and the result ranged from 130.36 to 209.33 for water absorption capacity, 2.00 to 2.34 for oil absorption capacity, 37.66 to 45.56 for emulsion property, 0.62 to 0.30 for bulk density, 55.73 to 60.36 for dispersibility and 0.47 to 0.63 for foaming capacity. Anti- nutritional factors like phytate, tannin, oxalate and alkaloids were determined, for raw samples 2.09, 2.36, 0.002 and 0.61 also for fermented samples ranged from 0.71 to1.94, 0.86 to 1.31, 0.004 and oxalate and alkaloid respectively. In the analysis of acute toxicity no toxic effects were observed.

Keywords: *Fermentation, Nutritional composition, Anti-nutritional, Phytochemical, Functional properties, Avena abyssinica, Acute toxicity.*

1. INTRODUCTION

1.1 Background

Oats (Avena) were domesticated much later than other temperate cereal crops such as wheat and barley. Probably it persisted as a weed in fields of these cereals for centuries before it was taken into cultivation. Oat seeds have been found in 4000-year-old remains in Egypt, but these were probably from weeds and not from cultivated oat. The oldest known cultivated oat remains were found in caves in Switzerland that date back to around 1000 BC. In industrialized countries oat grain mainly finds application as animal feed, especially for horses, but also for cattle, sheep, turkeys and other animals. Oats are an important source of livestock feed worldwide, both as forage and as a nutrious grain, and have played significant role in farming systems from domestication to the present time due to the versatile uses of the pomaceae family (Masood *et al*., 2008). Oatmeal are consumed traditionally as porridge or hard, flat cakes, or added to other dishes as a thickening. Nowadays, the main use of oats as human food is in breakfast cereals.

In Africa oat is harvested manually by sickle or scythe, for forage normally after heading, and for grain when the seed is in the hard dough stage, which is normally at the end of the rainy season. The harvest is left in the field for sun-drying and is subsequently threshed (grain crop) or piled (forage crop). Oats *or* Genus *Avena* comprises about 30 species such as *A.sativa(common oat) ,A.abyssinica and A,barbata* are some of the examples. *Avena sativa* probably evolved in central or northern Europe from wild *A.sterilis* . germplasm from south-western Asia. Nowadays Common oat is extensively cultivated in northern temperate regions, mainly in Europe and North America. In tropical Africa it is mainly grown in Ethiopia and Kenya. It is also cultivated in South Africa, Morocco, Algeria and Tunisia (Assefa *et al* 2003).

Common oat is mostly grown under cool and moist conditions in cool-temperate regions, mainly as spring-sown and to some extent autumn-sown crop. In tropical Africa it is mostly grown in mid to high altitude areas with an annual rainfall and maximum air temperatures. Common oat has been used as food and fodder since ancient times. Its grain is an ingredient in a wide range of food products including breakfast cereals, porridge, cookies, breads and muffins, crackers and snacks, beverages and baby foods. Among the species of Genus Avena; *A. abyssinica(wild oat) 'Sinar' in Amharic* is distinguished from the common oat (*A. sativa*) by the presence of two bristles at its lemma tip and probably originated from *A.barbata* .It is usually grown at high altitude area. It is generally long-day plant.It is native to Eritrea, Ethiopia and Yemen, and is cultivated for its grain in northern Ethiopia. It has been also tried as a crop in Tanzania and Algeria (Hanelt, 2001).

The traditional preparation of *A.abyssinica* for human consumption is more laborious than that of wheat since the grain has to be milled to remove the glumes, often after kiln-drying; then winnowed to obtain the "groats", which are the edible huskless grain, before any further milling or preparation. In Ethiopia *A.abyssinica* is made into 'injera' (pancake-like bread), 'tella' (local beer) and other products. The straw is used as forage, also serves as bedding for livestock, fuel and roofing material for traditional houses. A field sown for grain production can be used for grazing if rains are inadequate(Assefa *et al* 2003).

1.2 Statement of the problem

Developing African countries like Ethiopia are facing problems in overcoming food insecurity and applying poverty reduction mechanisms. Plants are major sources of food where more than 70 % of the population relies on agriculture, it remains the main vehicle for addressing food and nutrition security (Ochatt and Jain,2007). However only a few crop species are utilized for food production in our country. This is because the Cultivation of these crop is restricted to specific geographical location, centers of their origin and marginal lands (Assefa *et al*.,2003)

A.abyssinica is one of under-utilized crop with less importance in terms of production and consumption in Ethiopia (King 1979).The lack of attention and authenticated data on its potential for nutritional value make the crop under-exploited. However neglected or underutilized crops have the potential to play a number of roles in the improvements of food insecurity in our country(king 1979).So, studying the nutritional composition of these kind of cereal crops may fill the gap and misunderstanding towards its content and this may results in proper utilization of the crop as food consumption (king, 1979). This is important to provide strong effort to help feed poor and increase dietary diversity in Ethiopia.

1.3 Significance of the study

- The result of this research would reveal and significantly help in understanding the nutritional, antinutritional and phytochemical composition of *A. abyssinica*.
- The findings of this research encourage dietary use of the seed of this plant among societies across the country.
- The research will contribute to the knowledge of underutilized crops available in Ethiopia.

1. 4 Objective

1.4.1 General Objective

The general objectives of the research is to study the nutritional, phyto-chemical, physico-chemical, anti-nutritional and functional property, and effect of fermentation on these characteristics and also sensory acceptability and acute toxicity of *A.abyssinica flour*.

1.4.2 Specific Objectives

- To determine proximate composition, and antinutritional factors of raw and fermented *A. abyssinica* flour.
- To analyze the mineral content of raw and fermented *A.abyssinica* flour.
- To examine the phytochemical composition of raw and fermented *A.abyssinica* flour.
- To determine the functional properties and evaluate the sensory properties of *A.abyssinica.*
- To examine acute toxicity of raw *A.abyssinica* flour.

2. LITERATURE REVIEW

2.1 General information concerning common oats (*Avena*)

Oats are defined as species of cereal grains. The seeds of this plant have played an important role throughout history as both animal feed and food source due to their high nutritional content (Ripsin *et al*.,1992). It is one of the only cereals to be successfully grown in cooler northern climates, including Iceland, where many other cereal grains cannot grow. In fact, Russia, Canada, Poland, Austria, Finland, Germany, and the Ukraine are seven of the ten largest oat producing countries. According to FAO, these seven countries grew over 56% of the world's supply of oats (FAO 2009).

Historical evidence shows oats to be one of the last cereal grains to be cultivated by man, around 3,000 years ago in Europe, but it could be found growing wild in ancient China long years ago around 7,000 BC (Robert *et al*,. 1985). However, the common oat (*Avena sativa*) is believed to be derived from the accidental crossbreeding of two wild grasses, the common wild oat (*A. fatua*) and the wild red oat (A. *sterilis*). Oats most likely made their way by merchant caravans or enemy raiding parties to northern Europe where they became the food of choice for the Scots and Norsemen. However, it was the Romans who gave oats and other cultivated grain crops the term "cereal" (after the Roman goddess of agriculture Ceres). Oats became an American staple for the poor when they were brought to the "New World" in 1602 (Wetch, 2011).

The major growing region is concentrated mainly from the Midwest to the Northeast, but some production comes from Texas, California, and Oregon. However, with only 5% of the plant suitable for human consumption, it quickly began to undergo processing to increase its culinary uses, with the remaining going towards animal fodder .One possible reason people have been slow to embrace the oat is because their quickly go rancid, due to both the presence of natural fats and a fat-dissolving enzyme present in the grain (Hoover and Sendayake, 1996). One important note is that oat groats have the highest lipid content among the common cereal grains. Short processing times and steam treatments are typical pretreatments in industry to eliminate undesired enzyme activities (Mariotti *et al*, .2006)). In culinary terms, the term "oats" would be more accurately labeled "Oat products" since whole oats can be, and normally are, processed into many different forms.

Whole oat grain or groats, steel-cut oats, rolled oats or oatmeal, and instant oats are most consumed products (Mariotti *et al.,* 2006). Besides different processing forms, there are also a variety of raw oat products, such as whole oat groats, oat flour, oat germ, and oat bran. These varieties, as compared with the processed varieties, have a greater variation because the actual oat is broken down into its different forms.

In recent times, most of the world's oat production was used as animal fodder, with the last estimate of less than 5% of the world's crop being grown for human consumption. But due to increased knowledge about the nutritional benefits of oats, consumption has increased around the world. In addition, another growing segment for oats is in cosmetics due to the compounds in oat extract that have natural skin conditioners and anti-inflammatory properties (Emmons, 1999).

2.2 Nutritional composition of common oats

Oat has a well-balanced nutritional composition. It is a good source of carbohydrates and quality protein with good amino acid balance. Oat contains high percentage of oat lipids especially unsaturated fatty acid, minerals, vitamins and phytochemicals (Head *et al*, 2010).

2.2.1 Common oat starch

Starch constitutes about 60 % of oat grain. It is mainly a constituent of endosperm. There is considerable difference observed between the physicochemical properties of oat starch and other cereal starches. Differences in physicochemical properties are also observed in different cultivars of oat. These differences are probably due to differences in the magnitude of interaction between and among starch chains within the amorphous and crystalline regions of the native granules and by the chain length of amylose and amylopectin fractions of oat starch. Oat starch offers untypical properties such as small size of granules, well developed granule surface and high lipid content (Berski *et al*., 2011).

Hoover and Vasanthan, (1992) studied the characteristics of oat starches in light of their differences with other cereal starches. They reported that oat starches showed higher swelling factor, decreased amylose leaching, of a branched starch component and amylose during pasting process, higher peak viscosity and set back, low gel rigidity, greater susceptibility towards acid hydrolysis, greater resistance to α-amylase action and high free-thaw stability. However, wide range of differences has been observed among different cultivars of oats.

Starch has been classified into three fractions on the basis of digestion rate, rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Slow rate starch digestibility is important for human health to maintain balances of blood glucose levels. SDS is one of the most important fractions as it moderates the glycemic response and improves nutritional quality of the food (Ovando *et al.*, 2013). Resistant starch has been recognized as functional fiber. It is believed to perform an important role in digestive physiology. It escapes digestion and provides fermentable carbohydrates for colonic bacteria, like oligosaccharides such as fructo-oligosaccharides. They also provide benefits such as the production of desirable metabolites including short chain fatty acids in colon. Along with the therapeutic effects, resistant starch provides better appearance; texture and mouth feel than conventional fibers (Martinez *et al.*, 1999).

Resistant starch is naturally found in cereal grains and in heated starch or starch containing foods but is frequently destroyed during processing. RS doses of 20–30 g/day are required to observe physiological effects of RS consumption. However, this level is 3–4 times higher than the actual consumption reported for human diet (5–10 g/day); estimated RS intake among the United States population is 3–8 g/day. Most foods have RS content less than 3 g per serving (Murphy *et al.*, 2008). Oats contain significant amount of RS and other starch fractions. Approximately 7 % RDS, 22 % SDS and 25 % RS of the total starch has been reported in oats (Ovando *et al.*, 2013). Regular consumption of oat can be used to supplement these starches in diet.

2.2.2 Common oat protein

Oat is considered to be a potential source of low-cost protein with good nutritional value. Oat has a unique protein composition along with high protein content of 11–15 %. Cereal proteins have been classified into four types according to their solubility as follows: albumins (water soluble), globulins (salt water soluble), prolamins (soluble in dilute alcohol solution) and glutelins (soluble in acids or bases). Oat protein not only differs in the structural properties but also differs in distribution of protein fraction in comparison to other cereal grains. Other cereals such as wheat and barley have characteristic protein matrix which lacks in oat. In wheat and some other cereals, the storage protein is insoluble in salt solutions, while in oats, a large portion of salt water soluble globulins also belong to the storage proteins of the endosperm (Klose *et al*. 2009).

Oat contains lower quantity of prolamins (15 %) relative to the high amount of globulins (80 %) of the total oat protein. Prolamins (avenins) are low molecular weight fractions of oat proteins. These prolamins are soluble in 50–70 % ethyl alcohol or 40 % 2-propyl alcohol. Prolamins have high percentage of glutamine and proline and are low in lysine as compared to the other protein fractions (Capouchova *et al*., 2004). Avenins, a type of prolamins, have storage function similar to that of other cereal prolamins. Glutelin values are reported to be varying from 5 to 66 % of the total protein as they are difficult to be completely solubilized and are dependent on the extraction solvent and solvent concentration (Robert *et al*,. 1985). Of the total metabolically active proteins of oat, water soluble albumin accounts for most of the fraction. Albumins account for about 1–12 % of the total oat protein. In general, albumin and globulin have higher lysine content. Thus, oats are rich in lysine content compared to other cereals while they have rather lower content of glutamic acid and prolamin (Lasztity, 1996).

Celiac disease is triggered by the ingestion of gluten in gluten intolerant persons. Gluten is an alcohol soluble complex protein present mostly in wheat and other related cereals such as barley and rye. In individuals who are genetically susceptible, the ingestion of gluten causes an inappropriate small intestinal immune response characterized by villous atrophy and crypt hyperplasia (Fasano and Catassi,2001), resulting in malabsorption of protein, fats, carbohydrates, soluble vitamins, folate and minerals especially, iron and calcium. The only therapy available at present is to completely exclude gluten from the diet of the individual. Oat contains comparatively more favorable and nutritionally more valuable composition of protein fractions (Capouchova *et al.* 2004).

However, it has long been debated, whether oat can be considered safe for celiac patients (Ballabio *et al.*, 2011), while, Ripsin *et al.,* (1992), advocated the use of oats in celiac diet. The use of oats in gluten free diet depends on the composition of the protein fractions; albumins, globulins, prolamins (avenins) and glutelins. Prolamins together with glutelins forms the reserve protein located in the grain endosperm, which forms about 60– 70 % of the grain proteins of cereals. The prolamin fractions are less susceptible to hydrolysis and hence are also difficult to digest. The prolamins content in oats (10–15 % of the total protein) is rather low as compared to wheat (40–50 %), rye (30–50 %) and barley (35–45 %) (Capouchova *et al.*, 2004). Kumar and Farthing (1995) stated that avenins (oat prolamins) could be responsible for toxicity in the celiac patients only if oats are consumed in high amounts, as compared to rye and barley. Capouchova *et al*. (2004) reported that the amount of prolamins in oats varies with species, variety and time of cultivation and suggested that use of oats in celiac diet could be risky. However, recently European Commission regulation (EC) No. 41/2009 has included oats amongst permitted ingredients, if the gluten content does not exceed 20 ppm (mg/kg) (European Commission 2009).

Lapvetelainen and Aro (1994) reported that oats contain 78 % alkali-soluble, 11 % alcohol-soluble, 2.9 % salt-soluble protein fraction, 4 % residual protein and non-protein nitrogen (NPN). To broaden application of oat protein as a food ingredient, chemical modification was performed to improve solubility and further to increase good emulsifying and binding properties. These properties were found desirable for possible application in different low fat food products (Mohamed *et al*., 2009).

2.2.3 Dietary fibers

Dietary fibers (DF) are an essential part of the human diet. They consist of many substances of plant origin that are not digested in the human upper gastrointestinal tract. They include polysaccharides such as cereal β-glucan, arabinoxylans and cellulose. Dietary fibers are located in the cell walls of the grain. The outer layers, the seed coat and the pericarp contribute significantly to the insoluble dietary fiber content of the grain. According to The American Association of Cereal Chemists (AACC,2001), a dietary fiber is defined as the edible part of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation and/or blood glucose attenuation (AACC, 2001).

According to this definition, oat β-glucans are components of dietary fiber. As β-glucan is a plant polysaccharide resistant to digestion and absorption in the small intestine, it also attenuates both blood cholesterol and glucose. Schneeman (2001) suggested that dietary fiber regulates the rate of nutrient digestion and absorption and serves as a substrate for the microflora of the gut and promotes laxation.

The Codex Alimentarius Commission's committee on nutrition and foods in 2008 adopted a new definition of dietary fiber as "carbohydrate polymers with 10 or more monomeric units, which are not hydrolysed by the endogenous enzymes in small intestine of humans" (Codex alimentarius, 2010). Starch is not considered to be part of dietary fiber because it is hydrolysed by enzymes and is absorbed in the small intestine. Whole oats contain significant amount of dietary fiber, especially water soluble (1→ 3) (1→ 4) β-glucan (Peterson 2001). The β-glucan content in oat ranges from 2.3 to 8.5g/100 g (Flander *et al*., 2007).

The Food and Drug Administration (FDA) has accepted a health claim stating that a daily intake of 3 g of soluble oat β-glucan can lower the risk of coronary heart disease (FDA,1997). It is also known to reduce blood cholesterol level. Dietary fibers, particularly oat β-glucan has potential anti-cancerous property, as they reduce compounds which are causative agents of colon cancer, reduce blood cholesterol level and reduce blood pressure. The recommended dose of β-glucan for a single food is 0.75 g/serving (Flander *et al*., 2007).

2.2.4 Lipids

Oat is a good source of lipids. It contains much higher levels of lipids than other cereals which are excellent sources of energy and unsaturated fatty acids. The majority of lipids of oats are in the endosperm. The fat content of oat ranges from 5.0 to 9.0 % of the total lipid content. The lipid content in an intact kernel of oat stored for 1 year at room temp was found to be stable (Keying *et al.,* 2009). The stability is due to the protection from endogenous antioxidants such as tocopherols, L- ascorbic acid, thiols, phenolic amino acids and other phenolic compounds.

The lipids and other lipid associated compounds in the oat groat play an important role in the functionality of oat products. The high lipid content of oat provides an advantage when used for animal feed as it provides high energy along with good fatty acid composition. But when used as human food, this high lipid content provides fewer benefits, while leading to various processing problems such as poor flavor and excessive browning of toasted products. Along with lipids, oat contains considerable amount of lipases, which are capable of acting under low moisture condition. If not controlled, these lipases cause rancidity and short storage life for processed products of oat (Lehtinen *et al.,* 2003).

2.3 Antioxidant properties of oats

Oat has been widely shown to provide a vast range of human health benefits such as reduced symptoms of diabetes (Tapola *et al.,* 2005) and obesity (Zdunczyk *et al.,* 2006). The primary component of oat responsible for these health benefits is considered to be β-glucan, however phenolic compounds of oat and other antioxidant compounds also provide health benefits. Oats possess antioxidant capacity mainly due to presence of tocopherols, tocotrienols, phytic acid, flavanoids and non flavanoid phenoilc compounds such as avenanthramides.

2.3.1 Vitamin E

Antioxidants such as vitamin E are known to protect the body from damaging free radicals and play an important role in prevention of diseases such as cancer arthritis, atherosclerosis, cataract Packer (1991), Oat germ has high levels of tocopherols (a and c isomers), whereas tocotrienols are mainly concentrated in endosperm but, are absent in germ. The primary tocol of oat is α-tocotrienol but, small amount of tocopherols and their β homologs are also present. The total tocols ranged from 19 to 30.3 mg/kg. Out of the total tocols, α-tocotrienol & α-tocopherols account for 86 to 91 % respectively. Tocols are found to be stable in unprocessed groats for over 7 months of storage at room temperature, while processing of oats result in degradation of these compounds within 1 to 2 months (Peterson, 2001).

2.3.2 Phenolic compounds

Oat is a good source of phenolic compounds. These phenolic compounds may contribute to the functional and nutritional properties of the grain. Cereals account for phenolic compounds derived mainly from hydroxybenzoic and hydroxycinnamic acids. Early studies have shown that the phenolic acids in oat possess antioxidant properties both in vitro and in vivo (Peterson, 2001). The major phenolic acids in oats are ferulic, p-coumaric, caffeic, vanillic, hydroxybenzoic acid and their derivatives (Matilla *et al.,* 2005).

Traditionally, polyphenols are considered potent antioxidants. Emerging studies shows that polyphenols may have far more important effects in vivo such as enhancing endothelial function, cellular signaling and anti-inflammatory property. Oat hulls have not much uses in food but they contain significant amount of soluble ferulic acid; an avenanthramide antioxidant and also several other phenolic acids. The total free phenolic acid esters in oats are found to be low at about 8.7 mg/kg, whereas soluble phenolic acid esters account for 20.6 mg/kg and insoluble phenolic acids estimated to be about 57.7 mg/kg (Peterson, 2001).

2.3.3 Avenanthramides (AVAs)

Oats are known for a unique group of antioxidants reported among cereals known as avenanthramide (AVA) (Dimberg *et al.,* 1993; Meydani, 2009). There are abundant AVAs in oat, namely 2c, 2p & 2f, number 2 indicates 5 hydroxyanthranilic acid and letter c, p and f indicates the kind of hydroxylcinnamic acids as p-caumaric, caffeic and ferulic acids, respectively. Dimberg *et al.,* (1993) reported that AVAs have an antioxidant activity of 10–30 times greater than that of other phenolic antioxidants such as vanillin and caffeic acid. Preliminary studies indicated that the AVAs might possess anti-inflammatory and antiatherogenic properties, since they inhibit monocyte adhesion to human aortic endothelial cells and are presumed to inhibit release of proinflammatory compounds from macrophages (Liu *et al*., 2004). They are also involved in controlling the blood pressure, as they produce nitric oxide which dilates the blood vessels (Nie *et al.,* 2006).

2.4 Botanical and Genetic information of Avena abyssinica

A. abyssinica is a cereal which belongs to the tertiary Gene Pool of *A. sativa. A. abyssinica* is erect annual grass up to 1.5 m tall. It has leaves alternate, simple; leaf sheath long and loose; ligule acute, membranous; blade linear, flat, usually glaucous with inflorescence a terminal panicle 20–35 cm long, loose and open, the branches slightly rough. It has also, spikelet slender-stalked, pendulous, 2–2.5 cm long, 2–3-flowered, with the uppermost floret reduced or vestigial, non-shattering; glumes almost equal, narrowly elliptical, sharply acuminate, several-veined; lemma 1.5–2 cm long, smooth and glabrous or with a few bristly hairs near the awn insertion or margin, narrowly bifid, each lobe with 1 vein extended into an apical bristle 1–3 mm long, usually also minutely toothed at the base of the bristle, with slender, abruptly bent awn 2.5–3 cm long, arising from the back of the lemma; pale almost as long as lemma, bifid, 2-keeled, prickly hairy on the back; stamens 3; ovary superior, villous, with 2 stigmas and its fruit is caryopsis (grain) type (Assefa *et al*.,2003).

Figure 1: *A.abyssinica* crop

A. abyssinica is an annual plant. It flowers from Jun to July, and the seeds ripen from Aug to October. The flowers are hermaphrodite (have both male and female organs) and are pollinated by wind. It prefers dry or moist soil and can tolerate drought. *A. abyssinica* is globally assessed as least Concern as although it has a limited distribution, there is little evidence of significant threats to the species. This species would benefit from survey work to determine population size and trend. Evidence suggests that it is well conserved ex- situ but the species would benefit from in situ population monitoring program (USDA, National Genetic Resources program 2014). It grows on elevated basaltic plateaus above 2,400 meter and in cereal fields, mainly wheat and barley as a "tolerated weed" (Baum 1977).

Table 2.1 Taxonomic classification of oats (*Avena abyssinica*)

Taxonomy	
Kingdom	Plantae
Phylum	Tracheophyta
Class	Liliopsida
Order	Cyperales
Family	Gramineae
Scientific Name	*Avena abyssinica*
Common Name(s)	Abyssinian oat

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2.4.1 Genetic resources

A.abyssinica is a tertiary genetic relative of common oats *A.sativa* and so has potential for use as a gene donor for crop improvement, particularly for conferring pest resistance and drought tolerance to oats (USDA, National Genetic Resources Program 2014). The same source states that it is also used as a food source and in folklore medicine. This species is affected by crown rust; however, this is not thought to be a major threat Brink and Belly (2006). Other threats to this species are unknown.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

Glass wares, mill, digital balance and polyethylene bags and in proximate, antinutritional and functional property analysis instruments like; Kjeldahl, UV, blender, rotary evaporator, extraction cylinder, soxhlet and muffle furnace. were used from Food Science and Nutrition Laboratory of Addis Ababa university.

3.1.2 Chemicals

Analytical standard chemicals such as hydrochloric acid, petroleum ether, sulphuric acid, sodium hydroxide, nitric acid, ammonium hydroxide, calcium chloride, sulfosalicylic acid, calcium chloride potassium permanganate, iron chloride,vanillin,D-catechin,acetic acid,ethanol,aluminium chloride, sodium nitrite,sodium chloride, sodium bicarbonate and gallic acid were used.

3.1.3 Sample collection site

Sample (*Avena abyssinica)* was collected from Angolelana Tera around Chacha North Shoa Zone. Angolelana Tera is one of the woredas in the Amhara Regional state of Ethiopia. Chacha is the administrative center for Angolela ena Tera. (Fig.2) The distance of the study area is 81 km far from Addis Ababa.

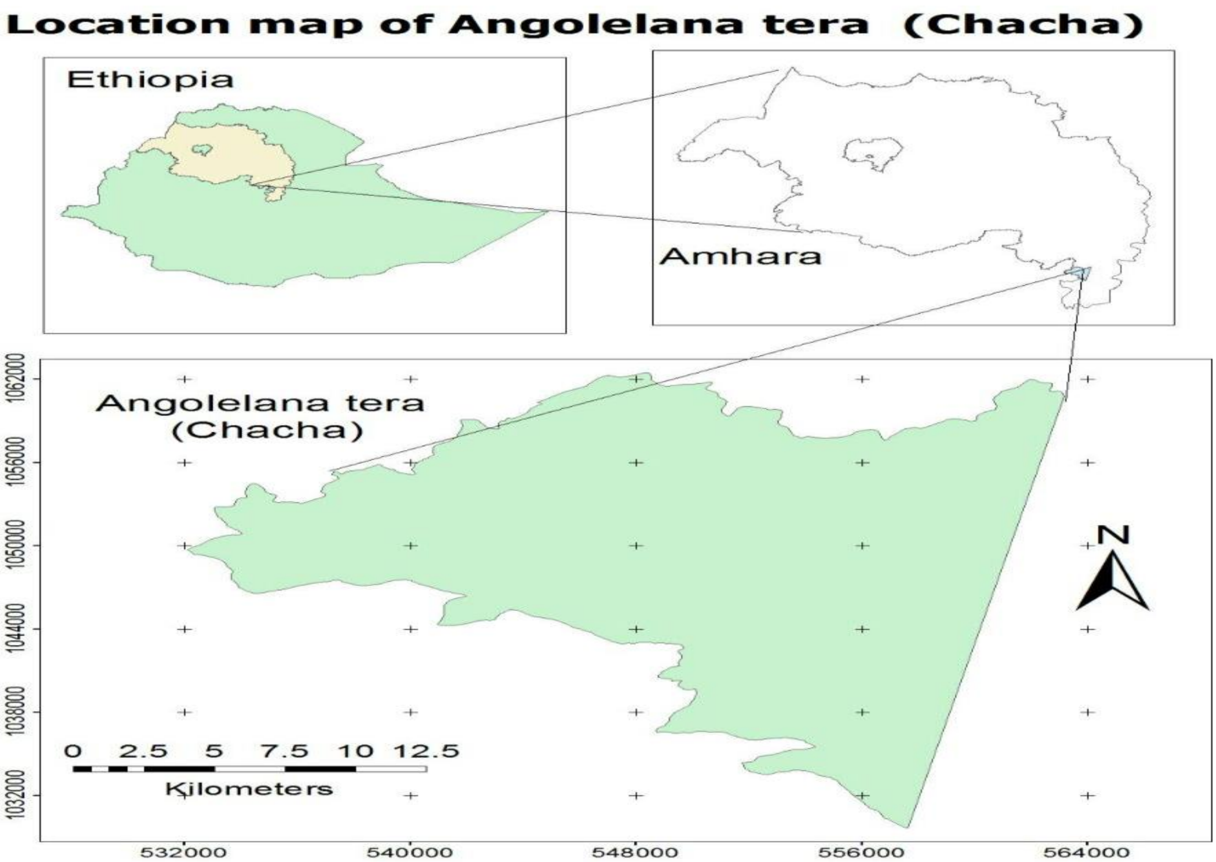

Figure 2 -location map of sample collection site (chacha).

3.1.4 Sample size

Total of about 15 kgs of unhulled *A. abyssinica* seed were collected from local market found in Chacha randomly and homogenized to be packed in polyethylene bag. Then the samples were transported to Center for Food Science and Nutrition Laboratory of Addis Ababa University within 2 days.

3.1.5 Sample preparation

For the preparation of flour, unwanted foreign materials (loose hulls and lighter feed grade seeds) were removed or cleaned followed by hulling process (removal of outer shell from the inner kernel) using a mill. Then the hull was removed by aspirator. Lastly clean kernel (groat) was milled using an electric mill and the powder was sieved through100 mesh sieve. Finally, the flour was packed by Polyethylene bag and stored until analysis.

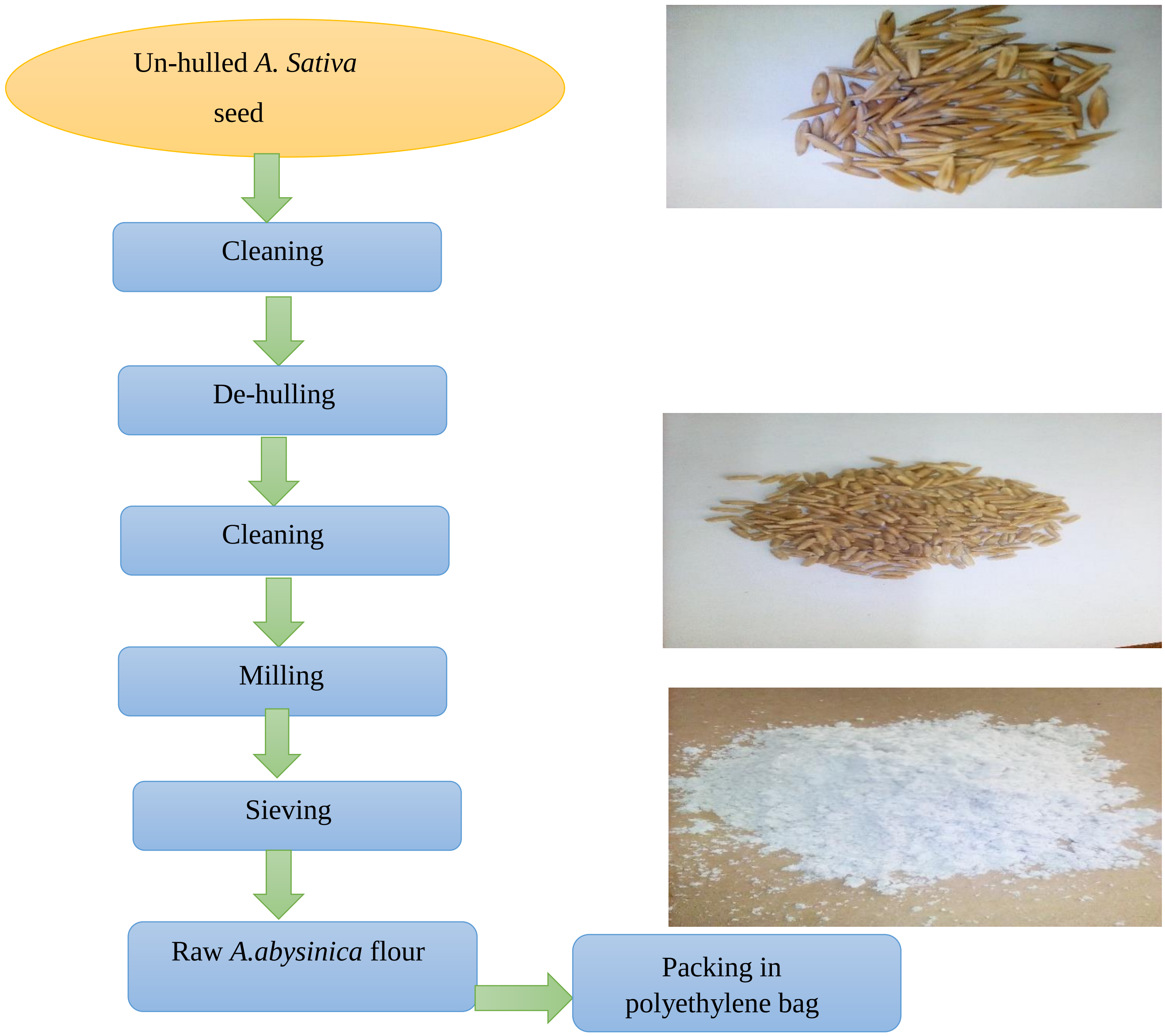

Figure 3- General flow diagram for preparation of flour from un-hulled *A.abyssinica seed.*

Fermentation process: Suspensions of *A.abyssinica* flour in distilled 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 (20-23 ◦C) for 24, 48 and 72hrs in 3 plastic containers. The fermentation water was decanted and samples were withdrawn and transferred to aluminum dishes after each fermentation time and dried in a hot air oven-drier at 50°C for 24h. Dried samples were ground with a miller (model-Multi-function comminutor,200) 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.

Experimental framework of the Research

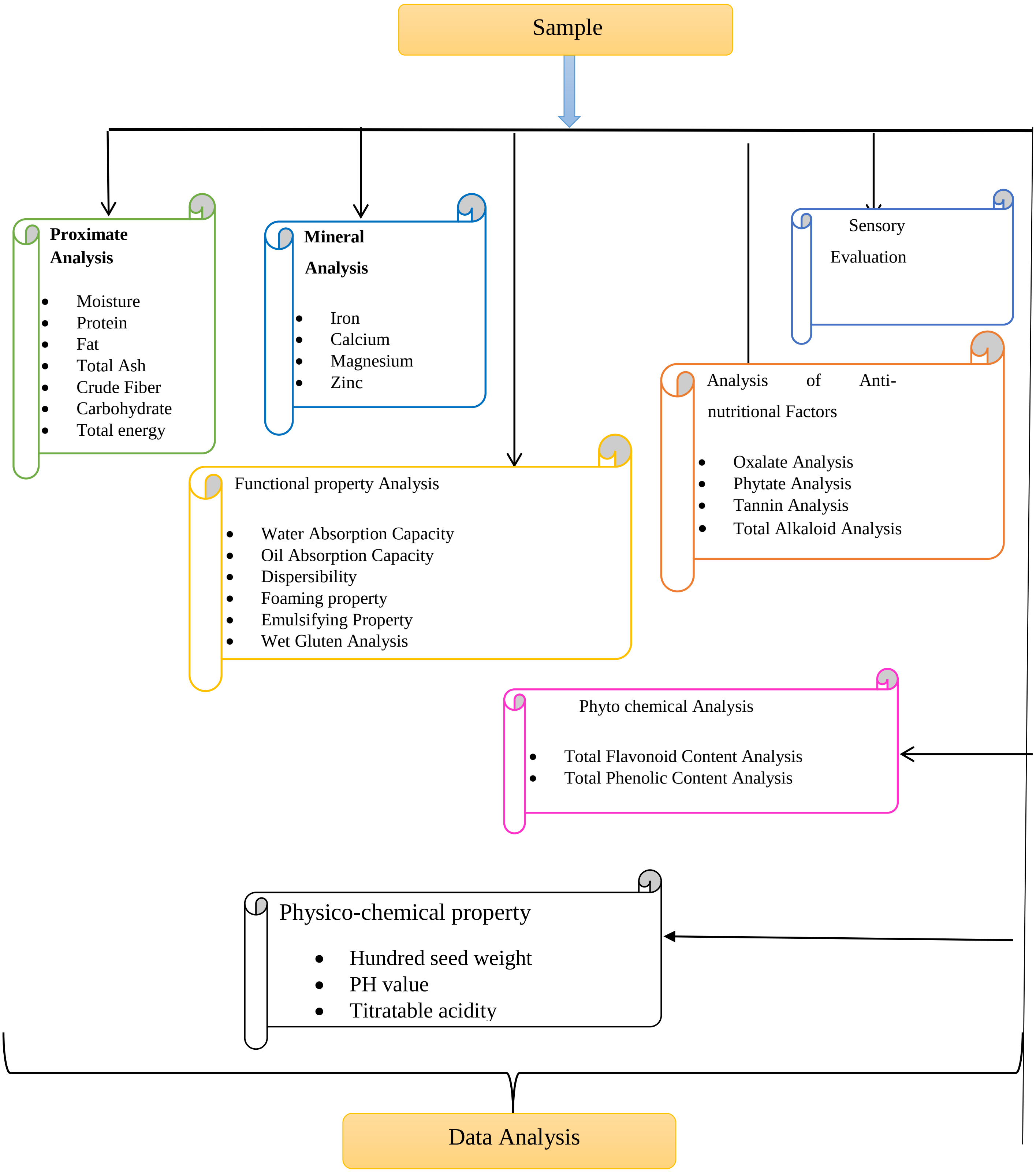

Figure4: - Experimental framework of the research

3.2 Methods

3.2.1 Determination of proximate composition

3.2.1.1 Moisture

Moisture content was determined according to (AOAC 2000 method no 962.09). Crucible was dried in an oven (Model DHG-9123A, Sweden) at 105ºC for 1hour and placed in desiccators to cool. Following this, the weight of each empty crucible was determined and 5 grams raw and fermented flour (sample) were added on it and allowed to dry at 105ºC for 3hours. Subsequently, samples were re-weighed after cooling in desiccators at room temperature. Then the moisture content was determined using the formula

Moisture content (%) = $\frac{W2-W3}{W2-W1} * 100$ ……………………………………Eq (3.1)

Where: - W1=weight Crucible

W2=weight of crucible and sample

W3= weight of crucible and sample after drying

3.2.1.2 Crude protein

Crude protein was determined by AOAC (2000 method number 979.09) using Kjeldahl protein analyzer instrument (FOSS Kjeltec - 8460 Analyzer unit, Sweden). About one gram of powder sample was weighed on analytical balance (Model ARA520, USA) and put it in Tecator tube then transferred into the tecator rack. Then 6 ml of acid mixture (5:1 of Conc. H_3PO_4: H2SO4) and 3.5 ml of 30% H_2O_2 mixed immediately with sample and 3.55 ml of 30% H_2O_2 was added into the digestion flask step by step. The tubes were shaked and watched out for a violent reaction. After this violent reaction was disappeared and added three grams of the catalyst mixture (0.5:100 of Se: K_2SO_4) was added into the digestion flask and stand for 5-15 minutes before digestion. The solution was then digested at 370 °C for 1 hr and cooled. After digestion was completed the distillation process was carried out using 50 ml of distilled water and the solution was then shaked to avoid precipitation of sulphate in the solution. Followed this, 25 ml of 40% sodium hydroxide was added to neutralize the acid and to make the solution slightly alkaline. A 250 ml of conical flask was placed and contacted 25 ml of boric acid, 25 ml of distilled water and indicator solution under the condenser of the distiller with its tip immersed into solution. The distillation was continued until a total volume become between 200 and 250 ml, borate ion was formed as a result of the reaction of the boric acid and the ammonia then; rinse the tip with distilled water before the receiver is removed. Finally, the solution was titrated with standard acid (0.1N HCl) until the green color changed to reddish color. The total N content was calculated by using the following formula: -

Nitrogen (%) = $\frac{(V2-V1)*N*14*100}{M}$ ……………………………………Eq (3.2)

Where: V1 = Volume in ml of the standard HCl used in the titration for the blank, V2 =Volume in ml of the standard HCl used in the titration for the test material, N = Normality of the standard hydrochloric acid, 14= Molecular weight of nitrogen and M= Weight of sample on dry basis

Protein (%, w/w) = %N * conversion factor …………………………Eq (3.3)

3.2.1.3 Crude fat

Fat content was determined using AOAC (2000 method no 925.09). Extraction cylinders were washed with hot water and dried in an oven for one hour at a temperature of 105ºC.Then cooled in desiccators. The bottom of an extraction thimble was covered with a layer of fat free cotton. Accurately 2gram sample were weighed in a thimble and covered with a layer of fat free cotton. The thimble was put in the extraction chamber (Soxhlet model XMTD-7000). Extraction cylinder was taken out of desiccator. The number (code) written on the extraction cylinder was checked and 50ml petroleum ether was added in it finally moved into the heating plank and extraction was continued for 4 hours.

The extraction cylinder was disconnected and put in the drying oven at 70ºC for 30 minutes then put in desiccator until it cooled for 30 minutes. Lastly,the extraction cylinder was re-weighed after it was taken out of the desiccators.Then finally, fat content was calculated from the formula ,

Crude fat, percent by weight= $\frac{W2-W1}{W3} * 100$ ………………………Eq (3.4)

Where; W1= weight of the extraction flask (g)
W_2=weight of the extraction flask plus the dried crude fat (g)
W_3= weight of sample (g)

3.2.1.4 Total Ash

Total ash was determined according to (AOAC, 2000 method no 923.03). porcelain crucible was cleaned and dried in a muffle furnace for 30 minutes at 550ºC.After the crucible was cooled in a desiccators. 2.5g of flour sample was placed on it and charred on a hot plate under a fume hood until smoke was ceased. Then the sample was ashed in muffle furnace (model -carbon lite Aston lane, HOPE sheffieled-5302RR England) at 550ºC for 5 hours. The ash was clean and white. Finally cooled and each crucible was reweighed with ash.

The ash content was determined with equation,

$$\text{Total ash (\%)} = \frac{M3-M1}{M2-M1} * 100 \text{ ……………………………….Eq (3.5)}$$

Where: M_1 = mass of crucible

M_2 = mass of crucible and sample

M_3 = mass of crucible and sample after ashing

3.2.1.5 Crude fiber

Crude fiber was determined by an official method of (AOAC 2016 method no 962.09) using two grams of sample. 200 ml of 0.255 N H_2SO_4 was added into a 500 ml beaker and placed on an electric heater. Then a suitable condensing flask (round bottom) filled with cold water and was kept over the beaker. After that the heater was switched on.

The beaker was heated to brought the acid (0.255 N H_2SO_4) to boiling stage. Then 2 g of substance was transferred to the boiled acid. The acid boiled and the sample is digested in acid. Boiling and digestion was continued for 30 minutes. After the end of 30 minutes boiling stopped and the condenser was removed.

Followed that set up of a funnel in a large conical flask was prepared for filtration. A linen cloth was fixed over the funnel and the contents from the beaker were transferred to the filtering funnel. After all the acid and acid digested residues were transferred to the linen cloth, the filtrate in a beaker was tested by adding two drops of filtrate over blue litmus. The blue litmus remained blue that means the residue was washed free of acid. After washing was completed the filter cloth was taken along with the residue, squeezed well (water was removed from the residue). Cloth was placed over porcelain slab. The adhering residue scraped gently from the filter cloth and the residue was kept in the center of the filter cloth.

Acid digested residue was then subjected to alkali digestion using 200 ml of 0.313 N sodium hydroxide solutions in 500 ml beaker. The alkali solution was brought to boiling stage by heating for 30 minutes by transferring acid digested residue. After 30 minutes, the condenser was removed; contents of the beaker were transferred to a filtering funnel. The residue was washed repeatedly with distilled water till it was alkali free.

This was tested by catching one or two drops of the filtrate over red litmus. It remained red and the residue was free from alkali. Once tested the residue was free from alkali then the cloth was squeezed to dry the residue well. The residue was transferred, without any loss, to a clean silica crucible.

The crucible was placed in preheated hot air oven (110ºC) over night. This was to drive off the moisture completely. After completely dried, the crucible was cooled in desiccator. It was weighed along with the residue. The crucible was heated with the electrical bunsen and the residue was ashed. Heating was continued till whitish ash was obtained. The crucible was cooled to room temperature and the weight of the ash was calculated using equation.

Percent of crude fiber = $\frac{a-b}{c} * 100$ …………………………………Eq (3.6)

Where: a=weight of crucible with dry residue

b= weight of crucible with ash

c=weight of sample (flour)

3.2.1.6 Utilizable carbohydrate determination

Total utilizable carbohydrate was calculated by difference with exclusion of crude fiber.

Total carbohydrate (%) = 100- (fat +fiber +protein +ash) ………………Eq (3.7)

3.2.1.7 Total energy in kilo calories

The gross energy (GE) content was determined mathematically using the following formula.

Gross energy (Kcal) = (9 x crude fat) + (4 x crude protein) +(4 x utilizable carbohydrate)..............Eq (3.8)

3.2.2 Mineral Analysis

Minerals were determined according to (AOAC 2000 method no 979.09) using 2.5 gm of sample on clean and dry crucible. Then the sample was charred to remove organic matter then ashed in muffle furnace at 550ºC for 5 hours. Three drops of 1M HNO_3 and few drops of deionized water were added to the sample in each of the crucible. The ash was digested by 3N and 6N HCl. The digested sample was filtered into sample bottles each using the Whatman filter paper (42mm) prior to analysis up to 50ml volume with deionized water.

For calcium determination 2.5ml of 10% $LaCl_3$ was added. Fe, Zn, Mg and Ca content in the sample were determined using Atomic Absorption Spectrophotometer (AAS) using air acetylene flame. AAS calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100g for all of the above minerals. Thus, reading was taken from the graph which depicted the metal concentration that corresponds to the absorption emission values of the sample and the blank. The metal contents were calculated by using the formula;

Mineral content (mg/100g) = $\frac{A-B*V}{100gw}$ ....Eq (3.9)

Where A= concentration of sample solution in ppm

B= concentration of blank solution from curve in ppm

W= weight of the sample

V= volume of extract

3.2.3Analysis of Anti-nutritional factors

3.2.3.1 Oxalate analysis

The oxalate contents of raw flour were determined using the method of (Iwuoha and Kalu 1995). This method involves the following three steps: digestion, oxalate precipitation and permanganate titration. For the digestion step about 2 grams of *A. abyssinica* flour were suspended in 190ml of distilled water contained in 250-ml conical (Erlenmeyer) flask; 10ml of 6M HCL was added and the suspension was then digested at 100ºC for 1hour. This was followed by cooling and then solution was made up to 250ml using distilled water before filtration.

In addition for oxalate precipitation, 125 ml of the filtrate was measured into a beaker and four drops of methyl red indicator was added followed by the addition of concentrated NH_4OH solution (drop wise) until the test solution was changed from its salmon pink color to a faint yellow color (pH 4-4.5). Each Portion was then heated to 90ºC, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90ºC and 10 ml of 5% $CaC1_2$ solution was added and stirred constantly. After heating, it was cooled and left overnight at 5º C. The solution was then centrifuged at a speed of 2500 rev/min for 5 min. The supernatant was decanted, and the precipitate completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution.

Finally, permanganate titration of total filtrate resulting from digestion of 2g of flour was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near-boiling, and then titrated against 0.05M standardized $KMnO_4$ solution to a faint pink color which persisted for 30s.The calcium oxalate content was calculated using the formula:

Oxalates (mg/100g) = $\frac{T*(Vme)(DF)*105}{(ME)*mf}$ …………………………………Eq (3.10)

Where T is the titer of KMnO4, (ml), Vme is the volume-mass equivalent in which 1 cm^3 of 0.05M KMnO4 solution is equivalent to 0.00225 g anhydrous oxalic acid. DF is the dilution factor VTA (2.4, where VT is the total volume of filtrate (300ml) and A is the aliquot used (125 ml), ME is the molar equivalent $KMnO_4$ in oxalate ($KMnO_4$ redox reaction)and mf is the mass of flour used.

3.2.3.2 Phytate Analysis

Phytate content was determined according to method described by (Vaintraub and Lapteva 1988). About 0.075 grams of *A.abyssinica* flour sample was extracted with 10ml. 2.4% HCl for l hour at ambient temperature and centrifuged at (3000 rpm/30 min) using (DYNAC II centrifuge, Clay Adams, division of Becton and Dikinson Company, USA). The clear supernatant was used for the phytate estimation. l ml of Wade reagent (0.03% solution of $FeC1_3.6H_2O$ containing 0.3% sulfosalicylic acid in water) was added to 3ml of the sample solution and the mixture was centrifuged. The absorbance at 500nm was measured using UV-VIS spectrophotometer. The phytate concentration was calculated from the difference between the absorbance of the control (3ml of water +1ml Wade reagent) and that of assayed sample. The concentration of phytate was calculated using phytic acid standard curve and results was expressed as of phytic acids in mg per 100 g dry weight.

Phytic acid in $\mu g/100 = \frac{(As-Ab)-(intercept)*10}{slope*W*3}$ ....Eq (3.11)

Where; As= sample absorbance

Ab= blank absorbance and W=weight of sample

3.2.3.3 Tannin analysis

Tannins were determined using the method used by (price *et al.*, 1987). About 0.25gm of *A. abyssinica* flour was added in a screw capped test tube containing 10ml of 1% HCl in methanol and kept in mechanical shaker at150rv/min for 24 hours at room temperature. After 24 hour of shaking, the tubes were centrifuged using (DYNAC II centrifuge, Clay Adams division of Becton and Dikinson Company, USA) at 1000xG for 5 min. One ml of the clear supernatant was taken and mixed with 5ml of vanillin HCl reagent in another test tube and this mixture was allowed to stand for 20 min to complete the reaction. After 20 minutes the absorbance was read at 500nm using a spectrophotometer (model). The concentration of tannins was calculated using D-Catechin standard curve and the result was expressed as D-Catechin equivalent in mg per 100g dry weight. Cyanide was determined by the AOAC official method.

$$\text{Tannin (mg/100g)} = \frac{(As - Ab) - int * 10}{100g * S * D * W} \text{ ……………………..Eq(3.12)}$$

Where: As = absorbance of sample solution

Ab= absorbance of blank solution

S= slope of the absorbance equation

D = density of the solvent and W= weight of the sample

3.2.3.4 Total Alkaloid analysis

The alkaloid content was determined gravimetrically by the method of (Haborne, 1973) as cited by (Shahidi, 2007). Five grams of each sample was dispersed in 50 ml of 10% acetic acid solution in ethanol. The mixture was shaked and allowed to stand for about 4 hrs before it was filtered. The filtrate was then evaporated to one quarter of its original volume on a hot plate. Concentrated ammonium hydroxide (NH_4OH) was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and the precipitate was washed with 1% ammonium hydroxide solution followed by drying in an oven at 60°C for 30 minutes. Then it was transferred in to desiccator to cool and then reweighed until a constant weight was obtained. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed. The experiment was repeated 3 times, the reading was recorded and the average of triplicate were calculated (Paoulos, 2009).

$$\text{\% Alkaloid} = \frac{Weight\ of\ alkaloid}{Weight\ of\ sample} * 100 \text{ …………...Eq (3.11)}$$

3.2.4 Analysis of phytochemical composition

3.2.4.1 Sample extraction

Extraction was based on the method described by (Blois, 1958). 5g *A.abyssinica* flour was extracted by 50ml of methanol at 25ºC at 150rpm for 24 hour using incubator shaker (ZHWY-103B) then filtered through Whatman No.1 filter paper. The residue was then extracted with additional 50ml of methanol and filtered as mentioned above. The extract was evaporated at 40ºC using rotary evaporator and re-dissolved in methanol at concentration of 50mg/ml and stored in refrigerator at 4ºCfor further use.

Figure5: Process of extraction for phytochemical test in the lab

3.2.4.2 Total Flavonoid Content

Total flavonoid content was measured by the aluminum chloride colorimetric method (Zhishen *et al* 1999). An aliquot of each extract (1 ml) was added to 10 ml volumetric flask containing 4 ml of double distilled water. Then 0.3 mL $NaNO_2$ 5% was added to the flask and after 5 min, 0.3 ml $AlCl_3$ [10%] was also added. At 6th min, 2 ml NaOH (1 M) was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed completely, and the absorbance level was measured versus prepared reagent blank at 510 nm (UV- visible spectrometer). Total flavonoid content was expressed as mg catechin equivalents.

Flavonoid content = $\frac{DE*V}{W}$ …………………………………………………. Eq (3.12)

Where DE=D-catechin equivalent (mg/ml)

V=total volume of the sample

W=sample weight (mg)

3.2.4.3 Total phenolic content

Total phenolics contents were determined according to the Folin-Ciocalteu method with slight modifications (siddhuraju and Becker, 2003). The extract (200 µL) was mixed with 1.5 ml of Folin Ciocalteu reagent previously diluted 10 times with double distilled water and allowed to stand at room temperature for 5 min. 1.5 ml sodium bicarbonate solution 60 g·L–1 was added to the mixture and after incubation for 90 min at room temperature, the absorbance level was measured at 725 nm using a UV-Visible spectrophotometer. Total phenolic was quantified by calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions 10 - 150 µg/ml in 80% methanol. The results was calculated as gallic acid equivalent (GAE) per one gram dry powder and reported as mean value ± standard deviation (SD). The total phenolic compound in the extract in gallic acid equivalent (GAE) was calculated;

Total phenolic content (C)= $\frac{\text{GAEc} \times \text{V}}{W}$ …………………………………Eq (3.13)

Where, GAEc=concentration of gallic acid equivalent(mg/ml) from curve

V=Total volume of the extract

W=sample weight (g)

3.2.5 Determination of physicochemical properties

3.2.5.1 Hundred seed weight

Measurement of hundred seed weight of *A. abyssinica* was carried out according to method stated in (ISTA, 2019). Triplicate measurement was taken and hundred seed weight was calculated as

HSW= $\frac{\text{sample weight}}{no\ of\ seed\ counted}$ x 100……………………………….Eq (3.14)

3.2.5.2 PH value

The pH of the raw and processed sample was determined according to the method of (AOAC 1984 method no 14.022 . About 10g of the samples were weighed in triplicates in 250ml beaker and mixed with some of distilled water and stirred for 10minutes. The pH of the sample was determined by dipping the electrode of Mettler Toledo AG (8603 Scherzenbach) in the mixture. The pH meter was calibrated using PH 4.0 and 7.0 buffers prior to determination of the pH of the sample.

3.2.5.3 Titratable acidity

Total titratable acidity of *A.abyssinica* flour sample was determined by method (pearson, 1973). About 5g of the flour sample was macerated for 30 minutes in a beaker with 15ml of distilled water as 1:3 ratio of the flour to water (W/V) ratio. A known volume of water is used for further dilution in order to hydrolyze all the acids in the sample. Before titration of the sample, water that is used for dilution purpose was titrated used as a blank. Three drops of 1% Alcoholic phenolphthalein indicator was added to water extract of the sample (dispersion). The dispersion was then titrated with standard base (0.1N NaOH) to phenolphthalein end point. The result of determination was reported as percentage lactic acid consuming definite volume of 0.1 N NaOH. The end point of the titration was reached when the white dispersion changed from a clear white solution to a faint violet colored turbid solution. Triplicate determinations were made in all cases. Finally, it was given that the amount of lactic acid in the sample was determined from the relation (l ml 0.l N NaOH 0.009008mg Lactic acid ($C_3H_6O_3$).

3.2.6 Determination of functional properties

The functional properties of *A. abyssinica* flours such as, water absorption capacity (WAC), oil absorption capacity, emulsifying property, foaming properties, dispersibility, wet gluten and bulk density were determined using different standard methods.

3.2.6.1 Water absorption capacity (WAC)

Water absorption capacity was determined according to (Beuchart 1977) whereby test tubes and crucibles were dried in an oven at 15 for 20 minutes and cooled in a desiccator. Then they were weighed and 1g weighed sample and 10 ml of water were added in each tube and stirred gently with a stirring rod for 30mins. The tube containing the mixture was centrifuged at 4000 rpm for 15 minutes and the supernatant was decanted into dry clean crucibles. Then crucibles were dried in an oven at 105^{0}c until the supernatant was dried off. The remained residue in the tube and the crucible after dried off the supernatant was weighed.

Water absorption capacity was expressed as the weight of water bound by 100 g dried flour. Water absorption capacity was calculated as

$$\text{WAC}= \frac{\text{Initial weight of the tube} - \text{Final weight of the tube (after absorption)}}{\text{Weight of the sample}} ------ (3.15)$$

3.2.6.2 Oil absorption capacity

Oil absorption capacity was analyzed according to method used by (Beuchart, 1977). 1g of sample flour was weighed in dry clean test tube and 10ml of refined corn oil with density of 0.92 g/ml mixed with flour. The content was stirred and centrifuged at 4000 rmp for 20 min. and the supernatant was decanted and measured using 10 ml cylinder. The difference in volume is the oil absorbed by the sample. Oil absorption capacity was calculated as,

Oil absorption capacity (ml) = (Volume of the added oil-Volume of decanted oil) --------eq(3.16)

3.2.6.3 Bulk density

A 5g flour sample was added to 50 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density (g/cm^3) was calculated as weight of flour (g) divided by flour volume (cm^3) (Okaka and Potter 1979)

Bulk density (g/cm^3) = $\frac{W1-W2}{volume\ of\ sample}$ ------------------------eq(3.17)

W1=weight of sample before tapping

W2=weight of sample after tapping

3.2.6.4 Dispersibility

Dispersibility was determined according to Mwasaru *et al* (1999). Ten g of flour sample was weighed and poured to 100ml measuring cylinder and distilled water was added up to 100ml volume then stirred and allowed to settle for 3 hours. The volume of settled particles was recorded. The volume of settled particles was subtracted from 100 and the difference was reported as percentage dispersibility.

% Dispersibility =100-volume of settled particle-----------------------------eq (3.18)

3.2.6.5 Foaming properties

Suspension was vigorously mixed for 2 minutes using a blender set at speed 2. The whipped mixture was transferred into 100ml graduated cylinder and the blender was rinsed with 10ml distilled water gently and finally added in to graduated cylinder. The foam volume was recorded after standing 30 minutes. And also, foam was determined as the foam volume remained after 8 hours. This was determined according to method used by Naczk *et al* (1985).

Foaming capacity (FC) (%) = $\frac{(v2-v1)}{v1} \times 100$----------------------- Eq (3.19)

Where: - (V1) initial solution volume

(V2) Final volume after mixing

3.2.6.6 Emulsifying properties

Emulsion activity and stability were determined according to the method used by Sosulski (1962). One-gram sample, 10 ml distilled water and 10 ml refined oil were added in calibrated centrifuge tube. The emulsion was centrifuged for 5 minutes. The height of emulsion layer (HEL) and the total height of the mixture or content (TC) were recorded (Triplicate measurements).

$$\text{Emulsifying property (\%)} = \frac{\text{height of emulsion layer(HEL)}}{Total\ hieght\ of\ mixture\ content(TC)}\ \text{x100}\text{---------------Eq(3.20)}$$

3.2.6.7 Wet gluten

Wet gluten determination was done by hand washing method (AACC, 2000). *A.abyssinica* flour (10 grams) was mixed with 4.8 ml of 2% sodium chloride solution and let it for 10 minutes in the container. Mixing and kneading until a rubbery, soft ball of dough with hand was continued. Washing and working the dough with hands under running cool distilled water squeeze gently procedures were preceded simultaneously. In this process, starch, water and salt soluble proteins were washed out via murky like water. Then the gluten was press dried between hands and rolled in to ball. The total weight of the gluten is defined as gluten quantity and wet gluten content of the sample was expressed as a percentage of the mass of the original sample . The wet gluten value was derived by the following formula:

$$\text{Wet gluten content (\%)} = \frac{total\ gluten}{wt\ of\ sample} x100\ \text{---------------Eq(3.21)}$$

3.2.7 Acute toxicity test

Acute toxicity test was carried out according to methods used by El sayed Ny *et. al* (1983) with little modification and internal SOP of Ethiopian Food and Medicine Administration and Control Authority.

A total of ten healthy and 20 days old male rodents (mice) were used for the test. The animals were kept in their cages for 5 days prior to the start of dosing and allowed for acclimatization to the laboratory conditions. The temperature of the experimental animal room was 20ºC. Lighting was artificial, the sequence being 12 hours light, 12 hours dark. For feeding, from 3-4g of *A.abyssinica* seed was used with 3-5ml supply of drinking water every day. A period of

24 hours allowed between the dosing of each animal. All animals were observed for 14 days. Animals were observed individually after eating the sample at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

The duration of observation was also determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded, with individual records being maintained for each animal. Additional observations were used to check whether animals continue to display signs of toxicity. Observations included changes in their restlessness, curling of hair, palpitation, tail straitening, etching and depression**.**

Individual weights of animals were determined before the test sample (*A.abyssinica*) was administered and weekly thereafter. Weight changes was calculated and recorded. At the end of the test surviving animals are weighed

3.2.8 Sensory Evaluation

Functional properties of flours play a significant role in food industry as their successful performance as food ingredients depend upon their functional characteristics and sensory quality, which they impart to various products (Lawless & Heyman, 2010).

Sensory evaluation was carried out by fifteen semi trained panelists of Food Science and Nutrition graduate students. The participants were 5 female and 10 male members, ages 29 to 37 with a mean age of 33 years. Nine point hedonic rating scale(1=Dislike extremely,2=Dislike very much,3=Dislike moderately, 4=dislike slightly,5=neither like nor dislike,6=like slightly,7=like moderately,8=like very much,9=like extremely) and preference test was used for the sensory analysis. The panelists were asked to score for sensory attribute like overall appearance, color, taste, odor and overall acceptability of gruel and porridge prepared from *A.abyssinica* and reference sample.

3.2.8.1. Sensory evaluation of gruel and porridge made from Avena abyssinica

Sensory evalution of *A.abssinica* was carried out by using *T.dicoccum* as acontrol group.becouse both species used in similar manner in the community and also belongs to the same family (Poaceae). The gruel was prepared from 100 gram flour of *A. abyssinica (sinar)* and *T.dicoccum(aja)* flour separately in one-liter cold water with 2g of sugar. The gruel was cooked for 45 minute and kept in thermos to keep its hotness until the judgment time was reached. Porridge was also cooked by mixing 125g of flour from both *T.dicoccum* and *A.abyssinica* flour separately in 500ml boiled water with 2g salt. During test time two spoon of porridge was placed in identical plastic plates and half cup of gruel were placed in similar glass cup to which the three letter codes (for the gruel -, *T.dicoccum represented by* BBA *and A.abyssinica* represented by BCA and for the porridge - *T.dicoccum represented by* BAA, *and A.abyssinica represented by* BCC) were attached on them that represent the test and reference sample. Finally panelists evaluated the samples at room temperature.

Sensory evaluation study was designed to determine preference test and overall acceptability of *A.abyssinica* gruel and porridge over *T.dicoccum* gruel and porridge. Preference test and rating acceptance tests using five sensory attributes with nine-point hedonic scale was used to evaluate the *A.abyssinica* sample. The analysis was performed at Laboratory of the Center for Food science and Nutrition, Addis Ababa University.

Figure 6: Panelists during sensory evaluation

3.3 Experimental design and statistical analysis

For the analysis of the results, all data were performed in triplicate and expressed as mean and standard deviation (SD). The data collected from the sensory tests were analyzed using SPSS® (version 23). Analysis of variance (ANOVA) was performed for the samples with comparison between *A. abyssinica* and emmer wheat. Significant differences were determined at $p < 0.05$.

4. RESULT AND DISCUSSION

In this study proximate composition, mineral composition, anti-nutritional factors, phyto-chemical, physico-chemical, functional properties, acute toxicity and sensory evaluation of raw and fermented *A. abyssinica* flour were studied.

4.1 Proximate composition of *A.abyssinica* flour

Proximate composition of raw and fermented *A.abyssinica* flour is presented in Table 4.1.

4.1.1 Moisture content

As it is shown in Table 4.1 the moisture content of raw *A.abyssinica* was 8.33% and moisture content of fermented sample at 24, 48 and 72 hrs was 9.96%, 10.5% & 11.38% respectively. All the results showed a significance ($p<0.05$) difference in moisture content. The fermented samples increased in the moisture content as the time of fermentation increased. Studies also showed that the moisture content of raw sample is lower than that of fermented samples. This might be due to absorption of water as fermentation proceeds (Ahmed *et al*, 2009). *A,abyssinica* flour samples had moisture content in the range of 8.33%-11.38.which is within the range for the effective storage of flour .Moisture content of 12–15.5% has been specified for cereal flour storage. Failure to store flour under this conditions leads to moisture absorption from the atmosphere, which eventually leads to caking (Whiteley 1971).

4.1.2 Crude protein

Crude protein content of raw and fermented *A. abyssinica* flour ranged from 11.46% to 11.90%.The fermented samples had higher protein value than the raw. (Table 4.1) The value of crude protein in raw *A. abyssinica* was 11.46% and for fermented sample were 11.90%, 11.78 % and 12.83% for 24, 48 and 72 hours respectively. In this study, there is no significant ($p>0.05$) difference in crude protein contents between raw and fermented sample for 24hrs and 48hrs. However, there was a significant ($p<0.05$) difference in crude protein content between raw, fermented for24 and 72 hours. The increase in protein content after fermentation has been attributed to the increase in nitrogen content released when microorganisms use carbohydrates for energy (Yagoub and Abdalla, 2007).

4.1.3 Ash content

Total ash value of *A. abyssinica* raw sample was 0.10% and samples fermented for 24,48 and 72hrs have 0.10 % 0.10% and 0.76% ash respectively (Table 4.1). There is no significant (p>0.05) difference in total ash content in all groups of samples except for 72 hours fermented sample. This difference in total ash content of fermented sample for 72 hours might be due to the synthetic activities of microorganisms during fermentation may increase in ash content and this possibly indicate a higher mineral content (Getachew *et al*., 2013)

4.1.4 Crude fiber

As indicated in Table 4.1 crude fiber content of raw *Avena abyssinica* flour was 7.92 %. Samples fermented for 24, 48 and 72 hours exhibited crude fiber content 7.40%, 7.24% and 7.11 % respectively. Raw *A.abyssinica* exhibited a statistically significant (p< 0.05) difference in crude fiber content compared to the fermented samples. Variation in fiber contents might be due to fermenting microorganisms ability to degrade the fiber in to short chain hydrolysable carbohydrate using their enzymes that are capable of hydrolyzing crude fiber into simple sugars, which the microorganisms use as their carbon source for the formation of other macromolecules (Oke,1967).

4.1.5 Crude fat

The crude fat contents of raw *A. abyssinica* were 7.33% and samples fermented for 24, 48 and 72 hours showed 7.00 %, 7.46% and 8.00 % respectively (Table 4.1). Fermentation had significantly (P< 0.05) increased the fat content of sample. An increased in fat content during fermentation was attributed by the action of fermenting microorganisms (Zhou, 1999).

4.1.6 Utilizable carbohydrate

Utilizable carbohydrate content in this study was determined by difference and the values were ranged from 72.34 to 73.68% (Table 4.1). For the raw 73.18% and fermented *A.abyssinica* sample at 24, 48 and 72hrs also exhibited values of 73.18%, 73.68%, 73.34% and 72.95% respectively. There was significant (P< 0.05) difference in all samples of carbohydrate content. As stated by Oho (2006), as fermentation time increased the carbohydrate content decreased. This is because of microbial activity hence a decrease in carbohydrate. Glucose released during fermentation is a preferred substrate for microorganisms fermenting the food and could partly explain the decrease in total carbohydrate after 24hr of fermentation (Oboh 2006).

4.1.7 Total Energy

As it is indicated in Table 4.1, the total energy content of *A. abyssinica* in raw and fermented samples ranges from 404.58 kcal/100 gm to 410.99 kcal/100 gm. The results were 404.58kcal/gm for raw and 405.10kcal/100gm,407.93kcal/100gm and 410.99kcal/100gm for fermented sample at 24,48and 72hrs. As the study showed that, there were no significant (p>0.05) difference in total energy of raw and fermented *A. abyssinica* for 24 hours. But there were asignificant (P< 0.05) different between raw,24hr fermented and 48hr fermented sample and also there was asignifcant difference between 72hr fermented sample and raw,fermented for 24 and 48hrs.On the other hand the total energy of *A.abyssinica* sample(showed in table 4.1) increased from raw with increased fermentation time(Inyang and Zakari, 2008).

Table 4.1 Proximate composition of raw and fermented *A. abyssinica* flour

Proximate composition	Raw flour	Fermented flour		
		24hr	48hr	72hr
Moisture content	$8.33 \pm .11^{d}$	$9.96 \pm .72^{a}$	$10.5 \pm .41^{c}$	$11.38 \pm .22^{b}$
Crude protein	$11.46 \pm .09^{b}$	$11.90 \pm .17^{b}$	$11.78 \pm .09^{b}$	$12.83 \pm .50^{a}$
Crude Fat	$7.33 \pm .25^{b}$	$7.00 \pm .00^{c}$	$7.46 \pm .05^{b}$	$8.00 \pm .00^{a}$
Crude Ash	$0.10 \pm .00^{a}$	$0.10 \pm .00^{a}$	$0.10 \pm .00^{a}$	$0.76 \pm .01^{b}$
Crude Fiber	$7.92 \pm .01^{a}$	$7.40 \pm .04^{b}$	$7.24 \pm .08^{ab}$	$7.18 \pm .01^{a}$
Total Carbohydrate	$73.18 \pm .30^{ab}$	$73.68 \pm .08^{a}$	$72.95 \pm .12^{b}$	$72.34 \pm .47^{c}$
Energy	$404.58 \pm .61^{c}$	$405.10 \pm .40^{c}$	$407.93 \pm .71^{b}$	$410.99 \pm .50^{a}$

All values were the mean of triplicate ±SD and

Values with different superscript are significantly different at p< 0.05.

4.2 Mineral composition of raw and fermented *A.abyssinica* flour

As it is indicated in Table 4.2, the mineral composition of calcium, magnesium, iron and zinc of raw and fermented *A. abyssinica* were studied.

4.2.1 Calcium

The calcium content for raw was 22.52mg/100gm and for fermented samples at 24, 48, and 72 hrs were 22.54, 22.53 and 22.54mg/100gm respectively. Based on the statistical analysis, there were no significant ($p>0.05$) differences in the calcium content of both raw and fermented *A. abyssinica* samples.

4.2 .2 Magnesium

The magnesium content in raw sample was 14.70 mg/100g while in fermented sample was 14.70, 14.71 and 14.70 mg/100g at 24, 48 and 72 hours respectively. This indicates that there were no significant ($p> 0.05$) difference in the magnesium content of raw and fermented *A. abyssinica* samples.

4.2 .3 Zinc

The values of zinc for *A.abyssinica* samples were ranged from 5.48.mg/100 g to 5.50 mg/100g. Zinc content of raw and fermented samples (24, 48 and 72 hours) were 5.50, 5.50, 5.80 and 5.49 mg/100g respectively. Based on the result from the statistical analysis used in this study, there were no significant ($p>0.05$) difference in zinc content of all samples of *A.abyssinica* samples.

4.2 .4 Iron

The values of iron as presented in Table 4.2 ranged from 8.77 mg/100g to 8.80 mg/100 g .Iron content for the raw and fermented samples (24,48 and 72 hours) were 8.80,8.80,8.77 and 8.80mg/100g respectively. Based on the result from the statistical analysis used in this study, there were no significant ($p > 0.05$) difference in the iron content of all *A. abyssinica* samples.

Generally, the result of mineral analysis indicated that, calcium is the most abundant mineral ranging from 22.52-22.55 mg/100g.And also it indicated that fermentation was not increased each minerals under study significantly .The finding of this study is in contradiction with study which described that, fermentation increases mineral content as microbes degrade carbohydrate and decreased phytate content (Parveen and Hafiz ,2003).

Table 4.2 Mineral composition of raw and fermented *Avena abyssinica* flour.

Mineral composition	Raw sample	Fermented sample		
		24hr	48hr	72hr
Iron	8.80±.02[a]	8.80±.01[a]	8.77±.01[a]	8.80±.02[a]
Zinc	5.50±.04[a]	5. 50±.05[a]	5.48±.01[a]	5.49±.01[a]
Magnesium	14.70±.03[a]	14.70±.02[a]	14.71±.01[a]	14.70±.02[a]
Calcium	22.52±.02[a]	22.54±.02[a]	22.53±.00[a]	22.54±.01[a]

All values were the mean ±SD;

Means in the same column with different letters are significantly different ($p<0.05$)

4.3 Anti-nutritional factors

The anti-nutritional factors such as phytate, tannins, oxalate and alkaloids were studied.

4.3.1 Phytate

The phytate content of *Avena abyssinica* sample were ranged between 1.94 to 2.09mg/100g for raw and fermented sample. The maximum reduction was observed at 72 hour for fermented sample (Table 4.6). The fermentation significantly ($p< 0.05$) reduced the phytate content of *A.abyssinica* flour. A study of Towo *et al*., (2006) stated that fermentation is known to cause reduction in pH. The optimal pH for cereal phytase is around 5.0, where phytase have high activity during fermentation this results in reduction of phytate.

4.3.2 Tannins

The tannin content for raw sample was 2.36 and fermented samples were 0.89, 1.31 and 0.86 mg/100g at 24, 48 and 72 hours respectively (Table 4.6). Fermentation significantly ($p <0.005$) decreased the tannin content of raw samples with increased fermentation period. Maximum reduction in tannin content was observed at fermentation time of 72 hours. Reduction in tannin content upon fermentation might be due to the activity of polyphenol oxidase or tannase of fermenting microflora on tannins (Molin, 2008).

4.3.3 Oxalate

As indicated in Table 4.6 fermentation reduced oxalate content of raw *A.abyssinica* flour to below detection limit. The values were 0.004 mg/100g for raw and 0.004 mg/100g for fermented sample at 24, 48 and 72 hours respectively. It indicates that, fermentation of *A. abyssinica* flour significantly ($p<0.005$) decreased oxalate content. Even if there was a decrease in oxalate content between the raw and fermented samples but there was no significant difference between fermented samples at different time. This reduction might be caused by effect of enzyme/acid hydrolysis of the oxalates during fermentation (Oke and Bolarinwa, 2012).

4.3.4 Alkaloids

The alkaloid content of raw and fermented *A. abyssinica* samples were indicated in Table 4.6 The values were 0.61 mg/100g for raw and 0.40 and 0.20 mg/100g for fermented samples at 24 and 48 hours respectively. The alkaloid content of *A.abyssinica* flour significantly ($p<0.005$) decreased when the time of fermentation increased. Fermentation decreased alkaloid content of cereals (Stray,1998).

Table 4.3- Anti-nutritional content of Avena abyssinica flour

Antinutritional	Raw	Fermented		
		24hr	48hr	72hr
Phytate	2.09 ± 0.00^{a}	0.71 ± 0.35^{b}	0.77 ± 0.46^{b}	1.94 ± 1.13^{a}
Alkaloid	0.61 ± 0.11^{a}	0.40 ± 0.00^{b}	0.20 ± 0.01^{c}	0.00 ± 0.00^{d}
Oxalate	0.004 ± 0.00^{a}	0.002 ± 0.00^{b}	0.002 ± 0.00^{b}	0.002 ± 0.00^{b}
Tanin	2.36 ± 0.01^{a}	0.89 ± 0.01^{c}	1.31 ± 0.44^{b}	0.86 ± 0.00^{c}

All values were the mean ±SD;

Means in the same column with different letters are significantly different ($p<0.05$).

4.4 Phytochemical composition of *Avena abyssinica*

4.4.1 Total Phenolic content

Total phenolic compound of raw sample extract was 374.6 mg GAE/g while the value for fermented samples were 401.40, 531.52 and 652.80 mg GAE/g at 24, 48 and 72 hour respectively. It shows that there were significant differences ($p < 0.05$) with an increment of total phenolic content in fermented sample. Similarly (Salar *et.,al* 2012)reported an increase in total phenolic content during the fermentation of whole grains through the activities of β-glucosidase, which is capable of hydrolyzing phenolic glucosides to release free phenolics.

4.4.2 Total flavonoid content

The Total flavonoid content of *A. abyssinica in raw* sample is ranged from 161.42 mg DE/g and in fermented samples was 168.93, 177.33 and 184 mg DE/ at 24, 48 and 72 hours respectively (Table 4.3). From this result it is shown that there were asignificant ($P<0.05$) differences with an increment of total flavonoid content of *A. abyssinica* in fermented samples. This might be due to microbial enzymes, such as amylase, glucosidase, esterase, lipase or invertase produced during fermentation which can hydrolyze glycosidase and break down plant cell walls or starch. These enzymes disintegrate plant cell well matrix and consequently facilitates the flavonoid formation (Hur *et al* 2014).

Table 4.4- phyto-chemical content of raw and fermented *A.abyssinica* flour.

Photochemical	Raw samples	Fermented samples		
		24hr	48hr	72hr
Total Phenolic content	374.6±.21^{d}	401.40±.36^{c}	531.52±.45^{b}	652.80±.62^{a}
Total Flavonoid	161.42±.12^{d}	168.93±.20^{c}	177.33±.35^{b}	184a±.52^{a}

All values were the mean ±SD and

Means in the same column with different letters are significantly different ($p<0.05$)

4.5 Physico- chemical properties

Physico- chemical properties of *A.abyssinica* such as seed weights, pH and titrable acidity were studied (Table 4.6)

4.5.1 Hundred seed weight

Hundred seed weight of naked *A.abyssinica* was determined. Accordingly the average hundred seeds weight of naked *A.abyssinica* was 3.11g.

4.5.2 pH value

The pH values for raw and fermented *A.abyssinica* sample ranged from 6.37to3.95 The change in pH from zero fermentation time to 72 hour resulted in a pH drop from 6.37to3.95 (Table 4.5). The pH of fermented samples had a significant ($p< 0.005$) effect in reduction of pH value of *A. abyssinica* samples compared with the raw. The decrease in pH during fermentation might be due to the production of organic acids by the microflora. Hetero fermenters were responsible to convert glucose to equimolar mixture of lactic acid, ethanol and carbon dioxide (Oke and Bolarinawa, 2012).

4.5.3 Titratable acidity

The titritable acidity values of the raw and fermented samples were expressed as percentage of lactic acid in the sample (Table 4.5). The value ot titratable acidity were 0.02 for raw and 0.11,0.16 and 0.20 for fermented sample at 24,48 and 72hrs. Fermentation had a significant effect ($P<0.005$) on increasing titratable acidity. This might be due to microorganism production of organic acid. As explained by (Oke and Bolarinawa, 2012).

concomitant with the drop in pH there was a rise in titratable acidity of cereal flours throughout the fermentation.

Table 4.5- Physico -chemical content of raw and fermented *A.abyssinica* flour.

physo-chemical property	Raw sample	Fermented samples		
		24hr	48hr	72hr
PH	6.37 ± 0.03^{a}	5.89 ± 0.01^{b}	5.70 ± 0.01^{c}	3.95 ± 0.05^{d}
TTA	0.02 ± 0.00^{d}	0.11 ± 0.00^{c}	0.16 ± 0.00^{b}	0.20 ± 0.00^{a}

4.6 Functional property

Functional properties were defined as properties that give information on how food ingredients behave in a food system (Vaclavik and Christian 2003). So, the functional properties of both raw and fermented *A. abyssinica* flour were determined. These are; water absorption capacity, oil absorption capacity, emulsifying property, bulk density, dispersability and gluten content.

4.6.1 Water absorption capacity

Water absorption capacity of raw and fermented *A. abyssinica* sample was shown in Table 4.4. The values are 209.33 for raw and 140.26, 135.3 and 130.36 for samples fermented for 24, 48 and 72 hours respectively. Fermentation significantly ($P<0.05$) decreased water absorption capacities as fermentation time increased. According to Adebowale and Lawal (2004), the decrease of water absorption capacity during fermentation could be related to the increment in fat content, as fat block the hydrophilic sites of amino acids and carbohydrate. So, water binding capacity is important indicator of whether the sample can be incorporated into aqueous food formulations. The baking quality of flours is a function of water absorption capacity (Shittu *et al.* 2008).

4.6.2 Oil absorption capacity

In this study, the oil absorption capacity of raw sample of *A. abyssinica* was found to be 2 ml/g while for fermented samples were 2.23, 2.25 and 2.34 ml/g at 24, 48 and 72 hours respectively. The result indicated that fermentation increased the oil absorption capacity significantly ($P>0.05$). This might be due to solubilization and dissociation of protein leading to exposure of non-polar constitutes within the protein molecule (Singh *et al*, 2013).As indicated in the result oil absorption was increased as the time of fermentation increased,studies suggested that, Oil absorption capacity is an important functional property that enhances the mouth feel while retaining the flavour of food products and also Absorption of oil by food products improves mouth feel and flavour retention, and this makes it an important property in such food formulations (Adebowale & Lawal, 2004).

4.6.3 Emulsifying activity

Emulsifying activity of raw *A. abyssinica* flour was 37.66 and for fermented sample 40.29, 40.29 and 45.56 at 24, 48 and 72 hours respectively (Table 4.4). All results of samples showed a significant difference (P<0.05) in emulsifying activity fermentation increased emulsifying activity of *A.abyssinica* flour. Studies indicated that increased emulsion capacity could be as a result of increased protein content because it's the protein being the surface active agents can form and stabilize the emulsion by creating electrostatic repulsion on oil droplet surface (Kaushal *et al.*,2012).

4.6.4 Bulk Density

As it was shown in Table 4.4, the bulk density values for raw *A. abyssinica* was 0.62 and for fermented samples the recorded result were 0.40, 0.34 and 0.30 at 24, 48 and 72 hours respectively. From this result, it is concluded that fermentation had a significant effect (P<0.05) on bulk density of *A.abyssinica* flour. The bulk density of the flours could be used to determine their handling requirement, because it is the function of mass and volume. The reduction in bulk density observed may be due to the breakdown of complex compounds such as starch and proteins a result of the modification that occurred during fermentation (Elkhalifa *et al*, 2005).

4.6.5 Dispersibility

The dispersibility of flour in water indicates its reconstitution ability. The result of dispersibility for raw sample was 60.36% while for fermented samples were 58.70%, 56.60% and 55.73% at 24, 48 and 72 hours respectively (Table 4.4). These values showed that fermentation had significant effect (P<0.05) on dispersibility and results in the decrease of measured values compared to raw sample. The fermented sample showed a decrease in dispersibility as the time of fermentation progressed. The values of dispersibility are relatively low for fermented sample than raw sample. Hence fermented sample cannot easily reconstitute to give fine consistency dough during mixing (Adebowale *et al.,* 2008).

4.6.6 Foaming capacity

The study also investigated the foaming capacity of *A. abyssinica* for both raw and fermented samples. For raw *sample* foaming capacity was 0.47 (unit) and for fermented samples foaming capacity was 0.48, 0.56 and 0.63 at 24, 48 and 72 hours respectively (Table 4.4). This result indicates that there is significant difference in fermentation ($P<0.05$) whereby increased the foaming capacity had been investigated when it was compared to the raw samples. An increase in the foaming capacity of fermented flour might be due to an increase in the percentage of protein content by fermentation process (Elkhalifa *et al., 2005*).

4.6.7 Wet gluten

Wet gluten is an elastic proteinous substance which is obtained after washing out the starch from wheat flour dough. Gluten content of *A.abyssinica* flour were determined by hand washing method and found to be zero (free). According to European commission *Avena* groups are officially concluded as gluten free (European commission regulation (EC) No. 41/2009) and thus found to be suitable for the celiac patients. Various reports suggest safety of oats to be included in the gluten free diet for children suffering from celiac disease (Hogberg *et al*., 2004; Holm *et al.*, 2006). Oats are considered as suitable in celiac disease. Hence, gluten free products such as pasta, biscuit and snacks have been developed for celiac patients from oats (Ballabio *et al*. 2011).Thus the current finding is in line with previous studies.

Table 4.6 functional properties of *A.abyssinica* flour

Functional property	Raw sample	Fermented sample		
		24hr	48hr	72hr
Water absorption capacity (ml/g)	209.33 ± 0.98^a	140.26 ± 0.75^b	135.30 ± 0.42^c	130.36 ± 0.35^d
Oil absorption(ml/g)	2.00 ± 0.00^c	2.22 ± 0.02^b	2.24 ± 0.02^b	2.34 ± 0.04^a
Emulsion property	37.66 ± 0.49^d	40.29 ± 0.52^c	42.93 ± 0.56^b	45.56 ± 0.59^a
Bulk density(g/ml)	0.62 ± 0.00^a	0.40 ± 0.00^b	0.34 ± 0.06^c	0.30 ± 0.01^c
Dispersibility (%)	60.36 ± 0.55^a	58.70 ± 0.26^b	56.60 ± 0.26^c	55.73 ± 0.86^c
Foaming capacity(ml/g)	0.47 ± 0.03^a	0.48 ± 0.03^a	0.63 ± 0.21^a	0.55 ± 0.09^a

All data are reported on dry weight basis, all values were the mean of triplicate ±SD

Means in the same column with different letters are significantly different ($p<0.05$).

4.7 Acute toxicity

In the results of acute toxicity test, experimental animals (mice) were used into two groups which are test and controlled group and the test groups were allowed to eat *A. Abyssinica* for 14 days and there was a follow up in the interval of seven days which means 3 times using different criterion (What are those different criteria; better to list here in bracket) that can indicate acute toxicity of the cereal. However, no symptoms where observed. As indicated in Table 6 and 7, individual animal data was provided. Additionally, all data were summarized to show number of animals used, animals displaying signs of toxicity and the number of animals dead during the test.

Concerning animals' condition during observation there was no death of animals recorded and none of them also showed any negative reaction. As indicated in Table 6 weight of test animals from day 1 to 7 and day 14 test groups were gained in weight even if their increment was in little amount it indicates *A. abyssinica* has no toxic effect, because weight gain is used as an indicator of toxicity in the method. Finally according to this study *A.abyssinca* doesn't Cause any toxicity. This is an indication that people can use this crop as stable food consumption.

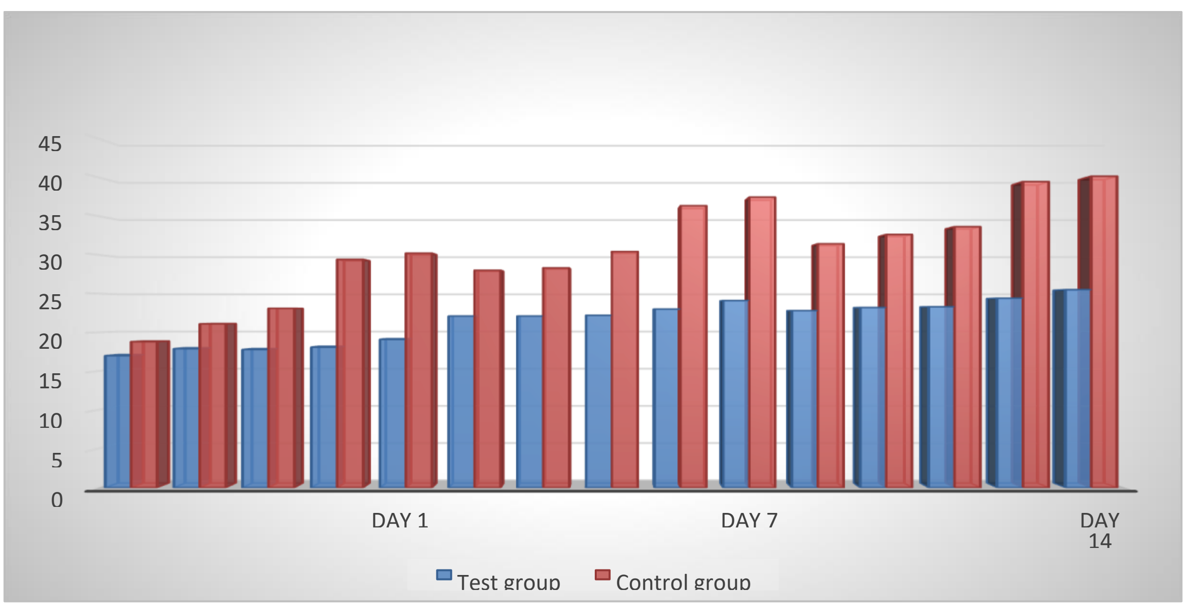

Figure 7: Weight of mice before, in between and after test

Table 4.7Animal condition during observation

Days	**Animal condition**													
	No of death		**Restlessness**		**Curling hair**		**Palpitation**		**Tail straightening**		**Etching**		**Depression**	
	Test	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	**test**	**control**
1	No	No	No	No	No	No	No	No	No	No	No	No	No	No
2	No	No	No	No	No	No	No	No	No	No	No	No	No	No
3	No	No	No	No	No	No	No	No	No	No	No	No	No	No
4	No	No	No	No	No	No	No	No	No	No	No	No	No	No
5	No	No	No	No	No	No	No	No	No	No	No	No	No	No
6	No	No	No	No	No	No	No	No	No	No	No	No	No	No
7	No	No	No	No	No	No	No	No	No	No	No	No	No	No
8	No	No	No	No	No	No	No	No	No	No	No	No	No	No
9	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10	No	No	No	No	No	No	No	No	No	No	No	No	No	No
11	No	No	No	No	No	No	No	No	No	No	No	No	No	No
13	No	No	No	No	No	No	No	No	No	No	No	No	No	No
14	No	No	No	No	No	No	No	No	No	No	No	No	No	No

4.8 Sensory acceptability

In this study, sensory evaluation was performed to evaluate the perception of consumers on *A.abyssinica* gruel and porridge. The sensory tests applied were preference test and rating acceptance test on the basis of appearance, color, taste, odor and overall acceptability. In this regard, the sensory attributes of emmer wheat (*T.dicoccum*) gruel and porridge were compared to *A.abyssinica* gruel and porridge.

4.8.1 Preference test for gruel

The panelists were able to detect difference in the sensory attributes between the two cereals. Sixty percent of the panelists preferred the gruel made from *A.abyssinica*. Consumer preference determines which product a consumer would choose/prefer (Lawless & Heymann, 2010).

Table 4.8: Frequency table for the preference test between *A.abyssinica* and *T.dicoccum*

Sample	Frequency (%)	Percent (%)
Avena abyssinica	9	60%
Triticum dicoccum	6	40%

4.8.2 Rating acceptance test for the gruel

Rating acceptance test was used to compute the mean values from the panelists' evaluations of the attribute ratings for appearance, color, taste, odor and overall acceptability of *A.abyssinica*. Panelists were asked to rate the attributes for both the *A.abyssinica* and *T. dicoccum* gruel using a 9-point hedonic scale with 1 being extremely dislike and 9 being extremely like. Data showing scores for the different sensory attributes of *A. abyssinica* is given in Table 4.9. In this study, the gruel made up of *A.abyssinica* flour had shown a higher score in all overall acceptability than *T.dicuccum*.

Table 4.9 Sensory scores for rating acceptance test on *A. abyssinica* and *T. dicoccum* gruel.

	sensory attributes				
Sample type	Appearance	Color	Taste	Odor	Overall acceptability
Triticum dicoccum	7.58± 0.26	7.50± 0.19	7.58 ± 0.14	7.92 ± 0.83	7.58 ± 0.19
Avena Abyssinica	7.33± 0.25	7.33± 0.28*	7.50 ± 0.23	7.83 ± 0.11	7.67± 0.14*

Mean ± SE with different asterisks along the same column are significantly different ($p<0.05$).
Hedonic scale: 9-extremely like; 1-extremely dislike

4.8.3 Preference test for porridge

In this study, the results for preference test between *A.abyssinica* and *T. dicoccum* indicated that the porridge made up of *T.dicoccum* preferred by 66.7% of the participants (Table 4.10). In this study, the results for preference test between *A.abyssinica* and *T.dicoccum* indicated that, 66.7% of the participants were preferred porridge made up of *T.dicoccum* as in indicated in Table 4.10 below .

Table 4.10: Frequency of the preference test between *A.abyssinica* and *T.dicoccum* porridge

Sample	Frequency (%)	Percent (%)
Avena abyssinica	5	33.7%
Triticum dicoccum	10	66.7%

7.8.4 Rating acceptance test for porridge

Rating acceptance test was used to compute the mean values from the panelists' evaluations of the attributes ratings for appearance, color, taste, odor and overall acceptability of *A. abyssinica*. Panelists were asked to rate the attributes for both the *A.abyssinica* and *Triticum dicoccum* porridge using a 9-point hedonic scale with 1 being extremely dislike and 9 being extremely like. Data showing scores for the different sensory attributes of *A.abyssinica* is given in Table 4.11. In this study, the porridge made up of *A. abyssinica* flour had shown a higher score in all overall acceptability than *T.dicuccum*

Table 4.11 Sensory scores for rating acceptance test on *A. abyssinica* and *T.dicoccum* porridge.

Sample type	sensory attributes				
	Appearance	Color	Taste	Odor	Overall acceptability
Avena Abyssinica	7.92 ± 0.22*	7.25 ± 0.21	7.50 ± 0.19	7.08 ± 0.26*	7.50± 0.19*
Triticum dicoccum	7.00± 0.27	7.08± 0.26	6.75± 0.27	6.83 ± 0.27	6.92 ± 0.22

Mean ± SE with different asterisks along the same column are significantly different ($p<0.05$).
Hedonic scale: 9-extremely like; 1-extremely dislike.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

A. abyssinica or Sinar its local name is used for food and to feed cattle in different parts of Ethiopia, especially in northern part of Ethiopia. This study tried to investigate nutritional composition, antinutritional factors, phytochemical composition, acute toxicity, and functional properties and sensory evaluation of *A.abyssinica*.

From the results of current study, it was indicated that *A.abyssinica* has sufficient amount of carbohydrate, crude fiber, crude protein and some amount of minerals like calcium, magnesium, iron with small amount of zinc. The result of this study also indicated lower content of phytochemicals such as flavonoids and total phenolic content when it is compared to other cereals in oats family. In the study of functional property as it was observed by hand washing method that *A.abyssinica* was gluten free.

This study also tried to address acute toxicity test and the result indicated that there was no sign of acute toxicity observed in *A.abyssinica* seed.From the result of sensory evaluation the gruel of *A.abyssinicca* is acceptable when compared to *T.dicucuum.*

5.2 Recommendations

- Food security being envisaged as a global concern, process upgradation of *A. abyssinica* derived products need to be further studied to ensure their proper utilization and thus contributing to the growing nutritional demands.
- Basic research is needed to determine important functional compounds in *A.abyssinica* and how to extract these components in fractions and how they can be incorporated in food products.
- Functional properties of flours play a significant role in food industry, this property are provided by proteins and carbohydrates. So in order to use it at industry level the slurries. Characteristics need to be studied.
- Although no toxicity is observed from these seeds acute toxicity study is warranted.
- *A.abyssinica* may be used as a component of composite flour in the preparation of traditional foods.

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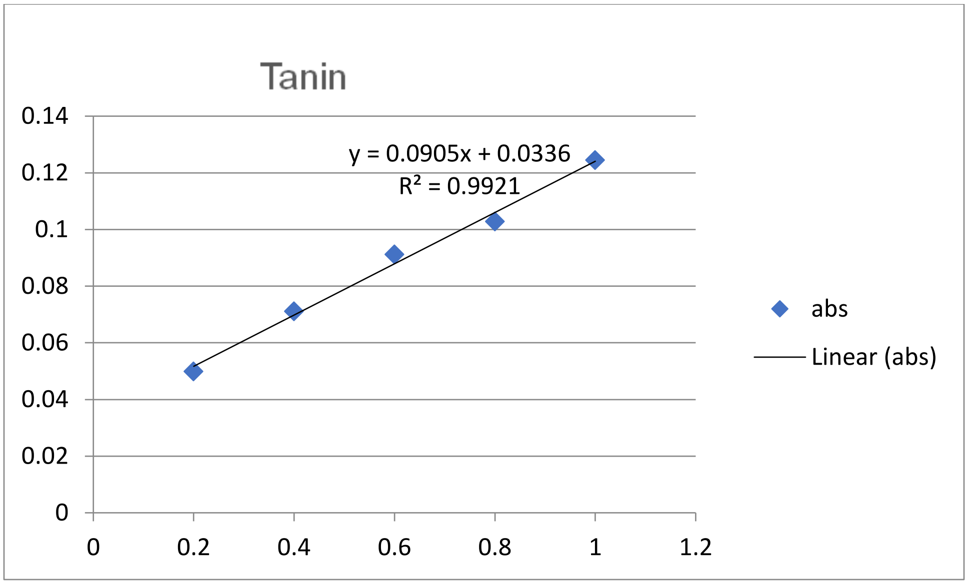
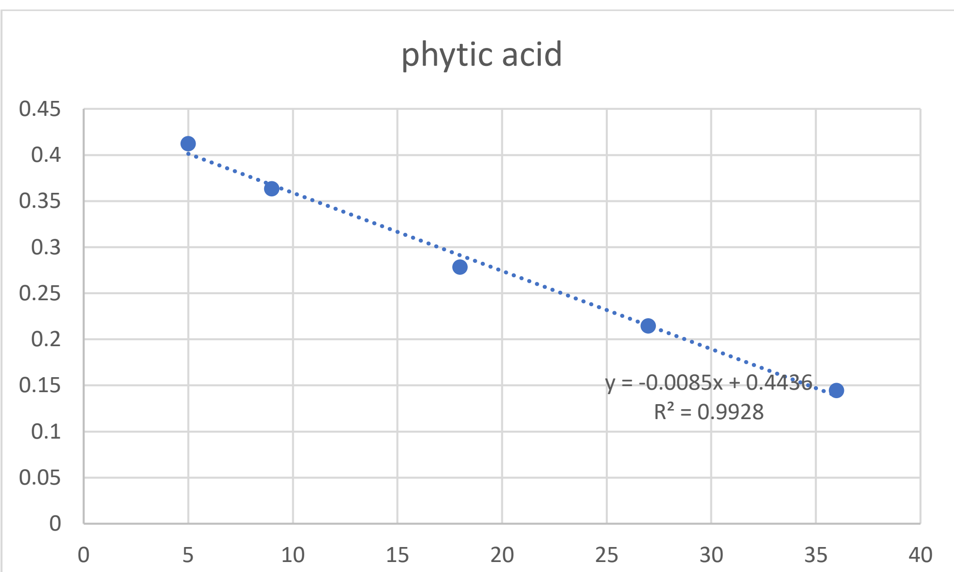
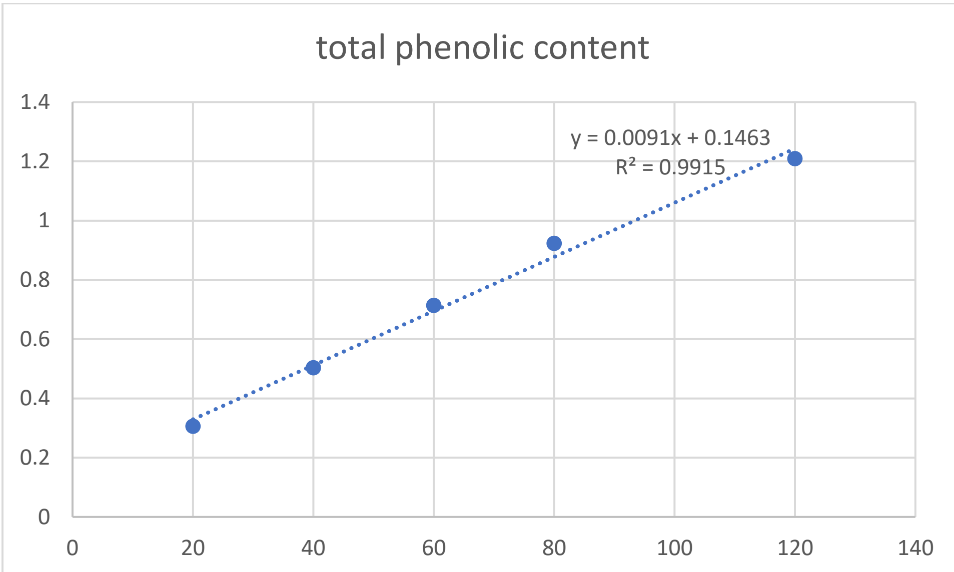
Appendix-1

1. Standard curve for anti-nutritional factors

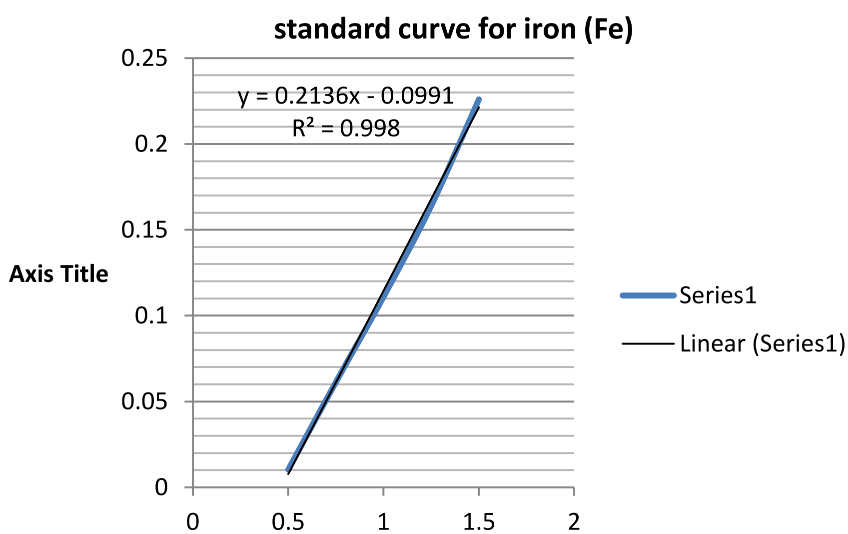




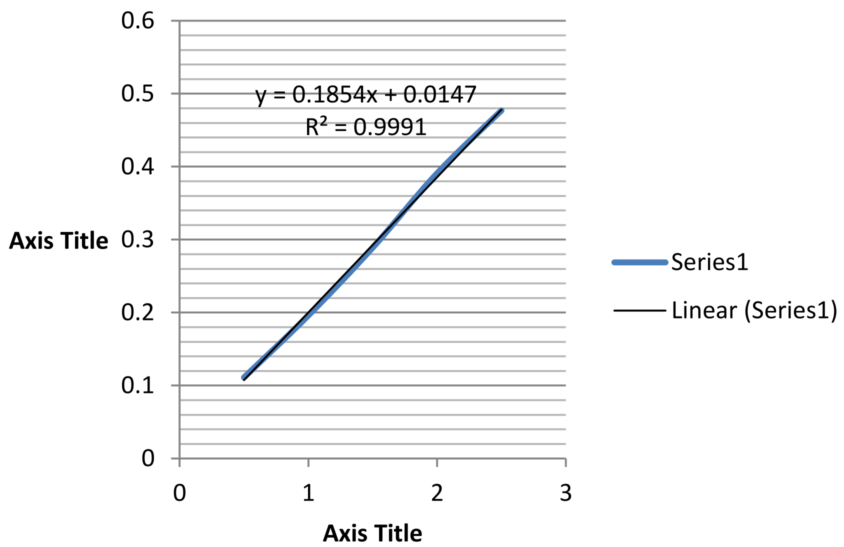
2. Standard curve for antioxidant



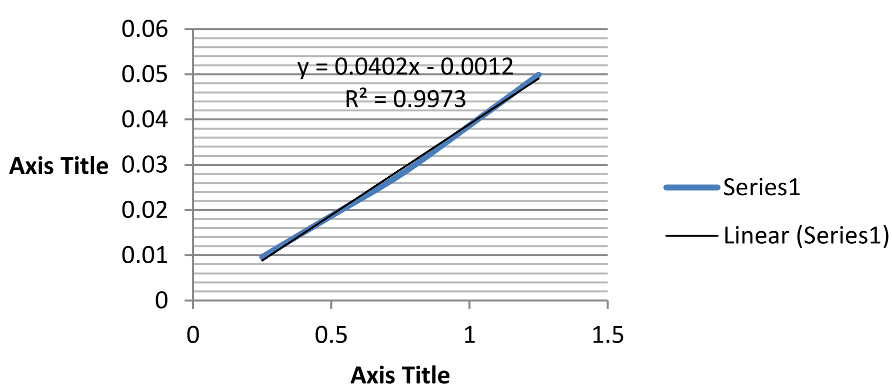
3. Calibration curve for minerals



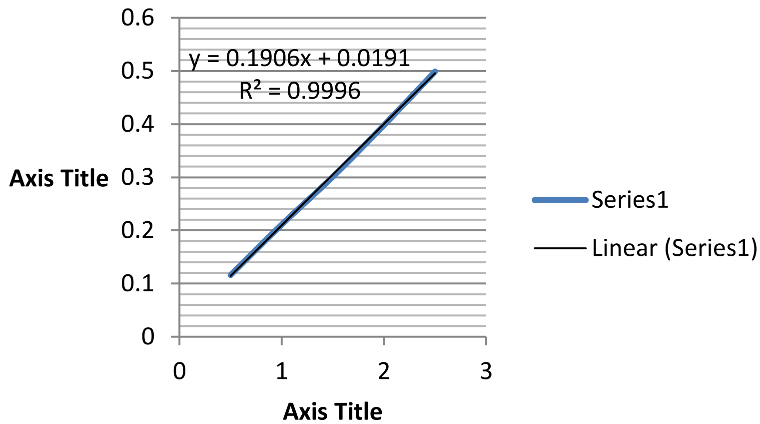
standard curve for calcium (Ca)



standard curve for zink (Zn)



standard curve for magnesium (Mg)



Appendix-2

Informed consent form for sensory evaluation panelists to participate in Descriptive analysis for Avena Abyssinica (sinar) porridge and gruel.

Dear panelist, You are invited to participate study involving Avena Abyssinica Porriage and gruel evaluation. The overall objectives of this study in to develop a descriptive list of term for the porridge and gruel to be tested. This product will be evaluation using asensory evaluation method known as descriptive analysis. You will be to oriented to identify overall appearance, color, taste, flavor, and overall acceptability of the sample. You will be asked to taste and expectorate the sample and to rate for intensity of each characteristics. Please you can put any comments you feel about the taste of the sample.

Your performance and date in this research is confidential. Responses are coded to be confidential and any publication or presentation of the results of the research will only include information about group Performance.

You are encouraged to ask any questions that you might have about this study whether before, during, or after your participation. Questions can be addressed to addressed to Zelalem Sisay (0913066121).

I understand the above information and voluntarily to participate in the study described above. I have been given a copy of this consent form.

Signature   Date

-------------------------------   --------------------------------------

Preference test

Date……………………   Time ………………………..

Dear participant,here are two samples for evaluation.Please examine left or right and put " sign on the sample you prefer.

Which sample do you prefer?

Please tick the appropriate box.

Code	BCA	BBA
Please tick		

You must make a choice

Thank you!

Indicate the degree of preference between the two samples.

Slightly--------------

Moderately-------------

Very much--------------

Extremely---------------

This is for sample coded as BBA

Score	Sensory perception	Sensory quality attributes				
		Overall appearance	Color intensity	Overall taste	Odor (flavor)	Overall acceptability
9						
8						
7						
6						
5						
4						
3						
2						
1						
Additional Comments(if any) …………………………………………………………………………………………………… …………………………………………………………………………………………………… **Thank you!**						

Appendix- 3

Ministry of Health

Ethiopian Food, Medicine and Health care Administration and Control Authority

Product quality assessment directorate

Toxicology case team

Toxicology Laboratory Analysis Certificate

Submission Details

Submitted by	A.A University College of Natural and Computational Science.
For the account of	Zelalem Sisay
Analysis No.	**TX-AT-0014/18**
Lab. Ser. No.	**0014/10**

Sample Details

Date sampled	**12/11/10 E.C**	*(if applicable) mfg.* Date	…………
		Exp. Date	…………………
Sample ID (Code)	**TX-AT- 0014/18**	Batch No.	………………………
Sample Description	Sinar		
Analysis Requested	**Acute Toxicity**	Method of analysis (test)	………………………

Analysis (Test) Results

The submitted sample of **"Sinar"** Was tested on laboratory animals under fourteen day's observation period. None of the animals were showed any sign, depression and symptom during observation period.

Comments:

We recommend further test to determine physiochemical and microbiological quality test and this test does not indicate toxic effect due to long term consumption.

Conclusion:

We didn't find any evidence of acute toxicity.

	Name	Sign.	Date
Analyzed by	Esubalew.Y	[signature]	Aug.01.2018
Reviewed by			
Approved by	Seyoum Wolde	[signature]	Aug. 01. 2018

Food Medicine and Health Care Administration and Control Authority of Ethiopia No 2

DIRECTOR, FOOD QUALITY CONTROL DIRECTORATE

EFMHACA,Product quality assessment directorate, Tel. 6541755/6/7/8Fax:251152139 P.O.Box 5681*Email: daca@telecom.net.et*