EVALUATION OF NUTRITIONAL QUALITY AND SENSORYCHARACTERISTICS OF *INJERA* FROM *TEFF(Eragrostis tef (zucc))* ENRICHED WITH PRETREATED FENUGREEK *(Trigonella foenum-graecum l.)* FLOUR

M.Sc. Thesis

By

Mihiret Getachew

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JIMMA, ETHIOPIA

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Mihiret Getachew

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I have completed my thesis research work as per the approved proposal and it has been evaluated and accepted by my advisers. Hence, I hereby kindly request the Department to allow me to present the findings of my work and submit the thesis.

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We, the thesis advisers have evaluated the contents of this thesis and found to be satisfactory, executed according to the approved proposal, written according to the standards and format of the University and ready to be submitted. Hence, we recommend the thesis to be submitted.

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DEDICATION

This thesis manuscript is dedicated to all my beloved family for their encouragement, continuous support for the success of my life and their affection and love.

STATEMENT OF THE AUTHOR

I, the undersigned, hereby declare that the thesis entitled “Evaluation of nutritional quality and sensory characteristics of *injera* from *teff(Eragrostis tef)* enriched with fenugreek (*(Trigonella foenum-graecum l.)* flour”is my actual work and all sources of materials used for this thesis have been duly acknowledged. This thesis is submitted in partial fulfillment of the requirements for M.Sc. degree at Jimma University College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author Mihiret Getachew was born on April 1990 G.C in Asendabo town. She attended and completed elementary school at Asendabo Elementary School (1996-2003). Upon completing her elementary school education, she attended her secondary and preparatory education in Asendabo high school from 2004 to 2007 GC. Mihiret Getachew started her university education at Jimma University in September 2008 G.C. and graduated with Bachelor of Science degree in Crop Science in June 2010G.C.

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LIST OF ABBREVIATION AND ACRONYMS

AAS	Atomic Absorption Spectrophotometer
AACC	American Association of Cereal Chemists
ANFs	Anti-nutritional Factors
ANOVA	Analysis of variance
AOAC	Association of Analytical Chemists
CRD	Complete randomized design
CSA	Central Statistics Authority
DPPH	2, 2-diphenylpicryl- hydrazyl
DZARC	Debrezeyt agricultural research center
FAO	Food and Agriculture Organization
GLM	General Linear Model
JUCAVM	Jimma University College of Agriculture and Veterinary medicine
SAS	Statistical Analysis Software
TA	Titratable acidity
USDA	United State Developmental Agency
WAC	Water Absorption Capacity

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ABSTRACT

One of the major problems associated with consumption of cereals alone may result in nutrient deficiencies. The use of pretreated seeds composite flour can improve the macro and micro nutrient deficiencies.However, scenarios indicate that the nutrient composition of teff is not comparable to legumes. Due to this the nutritional value for injera made from teff and pretreated fenugreek flour is increasing. This study was carried out to assess the effect of using differently processed fenugreek flour (raw, roasted, soaked, germinated and boiled) and substitution level on proximate composition, antioxidant activity and sensory characteristics of injera made from teff-fenugreek flour blends. Factorial experimental design was used with two factors arrangement (5x3) and analyzed by SAS 9.2 statistical software.Injera was made using teff flour incorporated in raw and pretreated fenugreek flour at the ratios of 100:0, 95:5, 90:10 and 85:15 for teff: fenugreek flour, respectively. Injera prepared from blend of teff with fenugreek (raw, roasted, soaked, germinated and boiled) at 5, 10 and 15% levels were compared with control (100% teff) for chemical composition, antioxidant activity and sensory properties. The results indicated that protein, crude fiber, calcium, zinc and antioxidant of the injera from blend of teff and fenugreek (raw, roasted, soaked, germinated and boiled) at 5,10 and 15% levels increased significantly at ($p<0.05$) with progressive increase in the fenugreek flour substitution. While the iron, carbohydrate and energy contents decreased significantly and higher at control sample. Mean values of protein, crude fiber, calcium, zinc, iron and antioxidant values were in the range of 12.95 to 9.36, 2.4, to 3.93, 77.28 to 103.41, 2.11 to 4.30, 20 to 25 and 34.19 to 57.69% respectively. The phytate and saponin content significantly decreased as increase in pretreated fenugreek flour than raw fenugreek blends samples. Processing enables that the antinutritional factors in the fenugreek couldn't hamper its nutritional value. Therefore, processing methods were effective to reduce the levels of antinutritional factors, thereby improving the bioavailability of zinc, iron and calcium.The result in sensory analysis gave overall acceptability of highest scores as 3.30, 4.00,3.73, 4.10 and 3.80 for raw, roasted, soaked, germinated and boiled teff-fenugreek blends injera at 5, 10 and 15% respectively. Generally, among all blends, injera from teff blend with 15% germinated and 15% soaked fenugreek flour had higher nutrients contents and antioxidant activity than other blend samples. Overall acceptability scores of injera were found highly acceptable at 5% (at par with control) and 10% levels blends.

Keywords Proximate composition, antioxidant capacity, *teff*, fenugreek, *injera*

1. INTRODUCTION

Teff (*Eragrostis tef*) is a cereal crop widely cultivated in Ethiopia mainly to process its grain flour into *injera* (a fermented, staple food for the majority of Ethiopians) (Bultosa *et al*., 2008). In its *injera* making features, *teff* grain is superior as compared to other cereal grains (Yetneberk *etal*., 2005).

Injera is a staple food for majority of Ethiopians. It is a fermented, pancake-like, soft, sour, circular flatbread (Bultosa, 2007). *Injera* is made from flour, water and starter *ersho* (Ashenafi, 2006). *Ersho* is a fluid saved from previously fermented dough.*Injera* can be produced from various cereals depending on availability and abundance of the cereals (Taylor, 2004a). It can be made from *teff* (*Eragrostistef*), wheat, barley, sorghum or maize or a combination of some of these cereals (Ashenafi, 2006). Good *injera* is soft, fluffy and able to be rolled without cracking. *Teff(Eragrostistef)injera* is getting popularity in Ethiopia as well as in the developed world because of its gluten free nature and being a whole grain product (Zegeye, 1997).

Teffinjera is a gluten free product and being fermented food from whole grain flour dough by lactic acid bacteria and yeast has pre- and probiotic potential. In many respects it favours toward complete nutrient supply with functional food character for consumers, particularly for celiac patients (Bergamo *et al*., 2011). *Teff* grain is favoured for its nutritional profile and is gaining popularity in Western countries (Kulp and Ponte 2000). *Teff* is a good source of carbohydrate, fiber (National Research Council 1996; USDA 2007), and contains more iron, calcium and zinc than other cereal grains, including wheat, barley and sorghum (Abebe *et al.*, 2007). *Teff* is an excellent source of essential amino acids, and it contains higher lysine content, an amino acid that is most often deficient in cereals, than other grains (Jansen *et al.*, 1962). Hence, the nutritional profile of *teff* indicates that it could be used in producing a healthy cereal product.

Fenugreek is a legume, originally from south Eastern Europe and western Asia, but grown now mainly in India and also in certain parts of Asia, Africa, Europe and the United States (Altuntas *et al.*, 2005). Its seeds are used as condiment in most parts of India, as a supplement to wheat and maize flour in Egypt and in Yemen; they are one of the main constituents of the normal daily diet (Uhl, 2000). Over 80 % of the total world's production of fenugreek seed is contributed by India,

one of the major producers and exporters of fenugreek seeds in the world. But widely cultivated in Egypt, Ethiopia and Morocco and occasionally in England (Davoud *et al.*, 2010).

Several health beneficial attributes of the spice, fenugreek, have been experimentally evidenced in recent decades, which have the potential of possible therapeutic application. In view of these promising beneficial physiological effects, fenugreek is understood to exert galactagogue, cholesterol-lowering, antidiabetic, digestive stimulant, antioxidant, gastroprotective and hepatoprotective effects (Srinivasan, 2006).

Fenugreek seeds can be a good supplement to cereals because of its high protein (25 %), lysine (5.7 g/16 g N), soluble (20 %) and insoluble (28 %) dietary fiber besides being rich in calcium, iron and beta carotene (NIN, 1987). In India seeds are used either boiled, pressure cooked, roasted or germinated, this basic processing is done to make seeds soft, palatable and to remove their bitterness (Mathur and Chaudhary, 2009).

However, Fenugreek seeds are bitter in taste due to the presence of bitter saponins, which limit their acceptability in foods (Valette *et al*., 1984; Udayasekhera *et al*., 1987). On the other hand, it has been possible to remove the bitterness from fenugreek seeds by employing various processing methods such as soaking, germination, roasting, etc. (Sharma, 1986; Shashi, 1997). As fenugreek seeds are rich in mucilaginous fiber and other dietary essentials, their use can be exploited as functional and nutritional foods as well as therapeutic agent. By keeping these facts in view of various value-added baked and extruded products from wheat-fenugreek flour blends have been developed (Hooda and Jood, 2003b).

In some parts of Ethiopia, women usually prepare *injera* by adding some fenugreek to *teff* to improve its baking quality. Because of this, the *injera* becomes softer and has a shiny appearance. Thus, women should be encouraged to continue this traditional practice and be made aware that their practice not only has the benefit of improving the baking quality of the *injera* but also of supplementing its protein content, especially lysine (Ketema, 1997).

As a spice, fenugreek adds nutritive value to foods as well as flavours (Brasch and Ulbricht, 2003); thus, it is used as a seasoning ingredient in products like artificial maple syrup and rum (Shankaracharya *et al.*, 1973). It is used in many domains, including medicine, nutrition,

beverages, fragrances, cosmetics, smoking, and for other industrial purposes (Djeridane *et al.*, 2006). This study aimed studying the effect of Pre-treated fenugreek (Raw, Soaked, Germinated, Roasted and Boiled) at different levels on the nutritional and anti-nutritional, mineral composition, antioxidant activity and Sensory property of *teffinjera.*

1.1. Statement of the problem

Cereal grains are somewhat limited in proteins, fats and vitamins (Blandino *et al.,* 2003). Blending of grains with nutritious seeds like fenugreek makes a nutritionally better food than does either alone. Among cereals *teff* is the major crop mostly used for making good quality *injera*. Nutritionally *teff* is better than other common cereals (wheat, maize and barley) but, it is not comparable with legumes. Blending of the two types of grains makes a nutritionally better food than does either alone. Fenugreek has been used as food spice and medicinal plant in many countries for centuries. It is a good source of essential amino acids, soluble and insoluble dietary fiber, and minerals. But, it contains phytochemicals such as phytates which binds minerals and interferes with their availability, and polyphenols and tannin that hinder the absorption of nutrients and the bioactive compound, bitter saponin that limits acceptability of fenugreek (Birhane, 2012).

There are different processing methods used to reduce those phytochemicals which limit the availability of different nutrients. These are germination, soaking and roasting (Hooda and Jood 2003a). In Ethiopia, there are various traditional foods with potential to be developed into functional foods for the benefits of Ethiopian consumers and for global competitive functional food markets. However, scientific investigations towards functional food development based on traditional foods are limited. *Injera* is consumed in Ethiopia as a major staple food; incorporation of *teff* with fenugreek seed may enhance its use with improved nutritional value and antioxidant potential. Hooda and Jood (2003b) reported the nutritional improvement of bread from wheat blend with fenugreek seed flour. Since peoples add fenugreek (mostly roasted) seeds in *teff* used for enhancing the sensory quality of *injera* by improving flavor, aroma, texture, etc. They need to know the appropriate concentration of fenugreek added into *teff*. Information on the characterization of *teffinjera* that are supplemented with fenugreek is limited. Therefore, in this study, the proximate composition and anti-nutritional composition, antioxidant capacity (*Teff*

with pre-treated fenugreek at 5%, 10% and 15% ratio), physical and sensory attributes of *injera* were evaluated.

1.2. Objectives

1.2.1. General objective

- To study and determine the nutritional quality and sensory properties of *teffinjera* enriched with fenugreek flour

1.2.1. Specific objectives

- To determine the nutritional and anti nutritional contents of *teffinjera* enriched with pretreated fenugreek flour of different percentage
- To determine the physical and mineral attributes of *injera* made from *teff*-fenugreek flourblends
- To determine the antioxidant capacity of *injera* made from *teff*-fenugreek flourblends
- To study effects of fenugreek flour ratio added on sensory property of *teff*-fenugreek *injera***.**

2. LITRATURE REVIEW

2.1. Production, Nutritional Composition and Trend utilization of *Teff* and Fenugreek

2.1.1. *Teff[Eragrostis teff (Zucc.)]*

Teff (*Eragrostis tef*) is an ancient tropical cereal that has its center of origin and diversity in the northern Ethiopian highlands from where it is believed to have been domesticated (Ketema 1997; Demissie, 2001). It is the most popular cereal grain for making*Injera*, which forms the traditional basic diet in Ethiopia, although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used (Bultosa, 2007). *Teff* has the largest share of area (23.42%, 2.6 million hectares) under cereal cultivation and third (after maize and wheat) in terms of grain production (18.57%, 29.9 million quintals) in Ethiopia (Central Statistical Agency of Ethiopia, 2008). The principal use of *teff* grain for human food is the Ethiopian bread *Injera*, a soft porous thin pancake with a sour taste (Yigzaw *et al.,* 2001; Schneider and Anderson, 2010**)**.

Nationally, *teff* ranks first in total cropland and quantity of produce among other cereals. The area devoted to *teff* cultivation and its productivity is increasing from year to year. In 2003-2004, it occupied about 2 million hectares (ha) which accounts for28.5% of the total cereal crops grown in the country. But in 2006/07 main cropping seasons, the total land allocated for *teff* production and yield obtained per hectare is 2.41 million hectare of land and 1.0414 ton/ha of grain yield respectively (Central Statistical Agency, 2007). Regionally, Amhara (778,202 ha) and Oromia (762,119.72 ha) have the largest acreage of *teff* followed by Southern Nations and nationalities and peoples region (133,882 ha) and Tigray (124,698.64 ha) (FAO, 2008b).

Teff injera

The principal use of *teff* [*Eragrostis teff (Zucc) Trotter*] is in *injera* production that constitutes the 70% of Ethiopians diet (Gambosa and Ekris, 2008). *Teffinjera* is the most common and the main staple food in much of the central, western and northern highlands of Ethiopia as well as among the urban community (Ashenafi, 2006). Wherever the soil type and rainfall patterns are suitable for cultivation of *teff, injera* from *teff* is more favoured than that from the other cereals (Bultosa, 2007).

2.1.2. Nutritional composition and antioxidant property of *teff*

Teff[Eragrostis teff (Zucc.) *Trotter]*, the tiniest grain in the world, is grown over a wide range of environmental conditions in Ethiopia, and has been utilized as the primary food and food supplements in Ethiopia (Gebremariam *et al.*, 2012).

Teff is known to have better nutritional value than common other cereal grains (wheat, barley, sorghum, maize and rice) because grain *teff* is always consumed as whole grain (Bultosa and Taylor, 2004). Grain *teff* bears about 11% protein, 73% carbohydrate (virtually all starch), 3% crude fiber (CF), 2.5% fat and 2.8% ash (Bultosa and Taylor, 2004). Grain *teff* is also rich with digestible-type proteins, and the essential amino acid profile is regarded as well balanced except lysine. Grain *teff* is recommended as functional food for celiac patients (Bergamo *et al.*, 2011), because it is gluten-free and offers in many respects more nutrient supply as it is consumed as whole grain often fermented as *injera*.

Teff is a good source of minerals, particularly iron. It has a very high calcium content and contains high levels of phosphorus, copper, aluminium, barium and thiamine. Zinc content of *teff* is similar to other cereal grains, such as barley, wheat or maize (Abebe *et al.*, 2007; USDA 2007).*Teff* is usually consumed as *injera* in which mineral inhibitors like phytates are reduced on fermentation (Abebe *et al.*, 2007). It is also rich in vitamins and is considered to be an excellent source of essential amino acids with higher levels than those found in wheat and barley (Forsido and Ramaswamy, 2011).

Antioxidants found in whole grain foods are polyphenols including phenolic acids and flavonoids, which are responsible for the high antioxidant activity(Ötles and Cagindi, 2006).Although antioxidants in fruits and vegetables have received more attention from researchers and contain high levels of antioxidants, grains and grain products contribute to the largest food intake according to the nutritional guidelines (Food Standards Agency, 2001), hence providing a considerable contribution to the antioxidant content in the diet.

Table 1 Nutritional composition of *Teff*

Component	Amount
Moisture Content(g/100g)	10.5
Crude Protein(g/100g)	11.0
Crude Fat (g/100g)	2.5
Crude Fiber (g/100g)	3.0
Ash(g/100g)	2.8
Carbohydrate(g/100g)	73.0
Energy(kcal)	357
Calcium(mg/100g)	165.2
Zink(mg/100g)	4.8
Iron(mg/100g)	15.7
Phytate (mg/100g)	389

Sources:USDA, 2007, Bultosa, 2007; Abebe *et al.,* 2007,Obilana , 2003

2.1.3. Utilization of *Teff*

In Ethiopia, it is traditionally grown as a cereal crop. It provides over two-thirds of the human nutrition in Ethiopia. The grain is ground into flour that is mainly used for making popular pancake-like local bread possibly with many eyes called *Injera* and sometimes for making porridge. Mixed with water and 'Ersho', the flour is allowed to ferment for a few days. 'Ersho' is starter, which is dough, saved from a previous fermentation and used as a starting fermentation in every new dough preparation. *Injera* is then, baked into large flatpancakes, done on a specialized electric stove or 'mitad' or 'Eele' on fire (Belay *et al.,* 2005).

Injera made from *teff* is traditionally consumed with a variety of stews such as wot, a sauce made of meat or roasted ground pulses like lentil, faba bean, field pea, broad bean and chickpea. Since wot, supplements the lysine deficit in *teff* and provides a better balanced diet, the traditional way of consuming *teff* with wot is wise. Because of its high mineral content, the need for *teff* grain is increasing to be used in mixtures with soybean, chickpea and other grains in the baby food industry (FAO, 2008b).

Fermentation is one of the oldest and most economical methods of producing and preserving food (Blandino, 2003). It is found to destroy undesirable components, to enhance the nutritive value, flavour and taste of the food, and to make the product safe from pathogenic micro organisms [6, 8]. In indigenous fermented foods, the microorganisms responsible for the fermentation are usually the microflora naturally present on the raw substrate (Ashenafi, 2006). Back slopping, that is, inoculation of the raw substrate with a small quantity of a previously performed successful fermentation is used to optimise spontaneous fermentation. This kind of a starter, which is a previously fermented product, is used not only to initiate the fermentation but also to accelerate the initial phase of fermentation and keep a uniform quality from batch to another (Sahlin, 2012).

2.2. Fenugreek

Fenugreek *(Trigonella foenum-graecum L.*) is an annual, self-pollinated plant from Leguminosae family with small seeds. Since ancient times has always been known as a medicinal herb (Slinkard, 2006). Its leaves are consumed as leafy green vegetables in India and are rich in calcium, iron, carotene B and other vitamins (Sharma, 1986). Fenugreek seeds are rich in protein, fixed oils and minerals; thus, it is quite nutritious. Fenugreek protein is also rich in lysine amino acid and in terms of supplying the protein requirements of the human body is similar to soybean protein (Hidvegi *et al.,* 1984).

Fenugreek is locally used as a pulse, spice and medicinal plant, and has a long history in Ethiopia. Even though the hectare is limited, the species has a considerable genetic diversity in seed colour, maturity and other morpho-agronomic characters (IBC, 2008). Characterization is essential for effective utilization of conserved germplasm. Characterization and preliminary evaluation on basic morpho-agronomic characteristics have been undertaken on about 70 percent of the crop germplasm accessions, including 34648 cereals, 8037 oil crops, 5355 pulses, 424 coffee and 360 accessions of fenugreek (IBC, 2008). Currently, 271,220.46 Quintals of fenugreek seeds were produced and about 51.09 per cent of fenugreek is used for household consumption, 15.19 per cent for seed and about 32.88 per cent for sale. The remaining 0.84 per cent of fenugreek is used for wages, animal feed and others (CSA, 2009/10).

2.2.1. Nutritional composition and Antioxidant property of fenugreek

Fenugreek seeds is used a spice and its leaves are used as a vegetable which is rich in vitamins and minerals. The seeds are protein rich; it is also an important source of diosgenin (Food Reference, 2004). Fenugreek seed contains 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans), 20-30% proteins high in lysine and tryptophan, 5-10% fixed oils (lipids), pyridine-type alkaloids, mainly trigonelline (0.2-0.36%), choline (0.5%), gentianine and carpaine, the flavonoids apigenin, luteolin, orientin, quercetin,vitexin and isovitexin, free amino acids, such as 4- hydroxyisoleucine (0.09%), arginine, histidine and lysine calcium and iron, saponins (0.6-1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin,yamogenin, tigogenin, neotigogenin), cholesterol and sitosterol, vitamins A, B1,C and nicotinic acid, coumarin compounds and 0.015% volatile oils (nalkanes and sesquiterpenes) (Blumenthal *et al.,* 1988).

However, fenugreek seeds are bitter in taste due to presence of bitter saponins, which limit their acceptability in foods (Birhane, 2012). It has been possible to remove the bitterness by employing various processing methods such as soaking, germination, roasting, etc. (Sharma, 1986). As fenugreek seeds are rich in mucilaginous fiber and other dietary essentials, their use can be exploited as functional and nutritional foods as well as therapeutic agent. Organoleptic (taste, flavor, color, texture, etc.) and nutritional characteristics have also been studied (Hooda and Jood, 2002).

There are many food and medicinal plants known for their antioxidant properties. Fenugreek (*Trigonella foenum-graecum*-known as ‘Methi’ in Hindi) is an important spice used in India and various other Asian, African and European countries. Its leaves, tender shoots and germinated seeds are consumed as vegetables. Fenugreek is credited with many medicinal properties and is also one of the oldest medicinal plants being used in many Asian and African countries for its health benefits. Its seeds, leaves and tender shoots show antidiabetic effects and are helpful in digestive disorders such as flatulence, dysentery, diarrhoea, dyspepsia, chronic cough and enlargement of the liver and spleen (Balch, 2003). In type 1 diabetic patients, supplementation of fenugreek in the diet lowers lipid peroxidation, induces hypocholesterolemia and hypoglycemia (Sharma *et al.*, 1990).

According to Bemihiretu *et al.* (2013) *injera*enriched with fenugreek appear to possess greater antioxidant activity when compared to non enriched *injera*. The reasons for the higher free-radical scavenging and total reducing ability of the enriched *injera* were due to the presence of fenugreek.

2.2.2. Utilization of fenugreek

Fenugreek is a chemurgical cash crop, usually cultivated as a break crop for cereal, as it is considered a good soil renovator. The whole plant is used as forage and vegetable, while the seeds (whole, powdered, in flour, or roasted) are used as human and animal food, spice, dyeing, flavouring, as well as for medicinal and industrial purposes (Petropoulos, 2002). Young plants and fresh tips of fenugreek are succulent and eaten as a salad, or cooked and generally served as a condiment in India and Egypt, as the fresh plant is very rich in vitamin C (207 mg per cent) (Saleh *et al.*, 1977).

The fenugreek seed is rich in protein, fixed oils and minerals and so it is nutritive and a tonic (Anonymous, 1994). In Sudan and Egypt the seeds are used in making beverages and in some countries the roasted seeds are used as a coffee substitute, probably because of the alkaloid trigonelline content, which is a basic constituent of the coffee seed. While in Ethiopia the seeds are prepared for infant feeding by boiling the whole seed (Fazli and Hardman, 1968). In North Africa it is mixed with breadstuff (Manniche, 1989), in Egypt also the seeds of the fenugreek are added to bread as a supplement of wheat and maize (Hidvegi *et al.*, 1984). In Yemen it is widely used every day by the general population (Manniche, 1989).

As a spice, fenugreek seeds add nutritive value to food, as well as flavouring and are used in soups and curries (Duke, 1986). Fenugreek seed is commonly used for seasoning purposes and as an ingredient of curry powder and sauces (Fazli and Hardman, 1968).

Table 2 Nutritional Composition of Fenugreek

Component	Amount
Moisture Content(g/100g)	6.3±0.34[a] , 13.7 [c]
Crude Protein(g/100g)	32.7±0.43[a], 27.20[b]
Crude Fat (g/100g)	4.8±0.16[a], 7.00[b]
Crude Fiber (g/100g)	6.0±0.07[a], 7.96[b]
Ash(g/100g)	3.7±0.25[a],4.28[b]
Carbohydrate(g/100g)	46.1±1.14 , 53.56[b]
Energy(kcal)	1495.85[b]
Calcium(mg/100g)	160[c] ,70.5±0.68g[a]
Zink(mg/100g)	5.7±0.34g[a],
Iron(mg/100g)	11.6±0.5g[a], 14.1[c]
Phytate (mg/100g)	552.3 ±2.52[a],
Saponin(mg/100g)	4581.3[b]
Antioxidant activity (%)	18.1[a],

Sources:[a]Pandey and Awasthi 2013,[b]Hussein *et al.,* 2011, [c] (Gopalan *et al.,* 2004)

2.3. Major Antinutritional factors in *Teff* and Fenugreek

2.3.1. Phytate

Phytates are a common constituent of cereals and legumes. It is the primary form of phosphorus storage in seeds and accounts for 60-90 percent of the total phosphorus (Schlemmer *et al.,* 2009).

The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Minerals of concern in this regard would include Zn 2+, Fe 2+ / 3+, Ca 2+, Mg 2+, Mn 2+, and Cu 2+ (Vucenik, 2003). Its highly negatively charged structure at a wide range of pH values makes it very reactive with other positively charged ions such as minerals, forming insoluble complexes which are less available for digestion and absorption in the small intestine. This is the main reason why PA has traditionally been considered as an antinutrient. The adverse effect of PA in mineral availability depends on a number of factors including the concentration

of PA and the strength of its binding with different minerals. For example, zinc (Zn) forms one of the strongest mineral complexes with PA (Evans and Martin, 1988). PA can also react directly with the positively charged group or indirectly with the negatively charged group of the proteins mediated by a positively charged mineral ion such as calcium. It can bind with starch either directly by hydrogen bonding with the phosphate group or indirectly through the proteins to which it is associated with. The formation of these complexes is likewise thought to reduce the solubility and digestibility of the proteins or starch and several in-vitro Carnovale *et al.* (1988) studies have indeed shown reductions in protein digestibility by PA and in-vivo (Atwal *et al.,* 1980).

Teff grain contains less than 1% (528-842mg/100g) phytic acid and other inositol phosphates, which are strong inhibitors of Fe and Zn absorption. The amount of phytates in *injera* is considerably reduced to 35-76 mg/100g (91-93% destruction) due to fermentation and the acidity nature of *injera* (Melaku *et al.,* 2005). *Teff*'s phytate content is comparable to values reported for wholegrain cereals (Schlemmer *et al.,* 2009). Such high values in phytate are likely to impair the absorption of iron and zinc (Hurrell and Egli, 2010). In legumes a considerable proportion of phosphorus (60 to 80%) is formed as phytic acid or complexed with protein (Emami and Tabil, 2002). Birhane, 2012 found that the phytate content in fenugreek was 76.11 to 111.08 mg/100g.Phytic acid content will vary with genotype, climate, type of soil and year (Muzquiz and Wood, 2007).

2.3.2. Saponin

Saponins are a diverse group of compounds commonly found in legumes, e.g. chick peas, soya beans, lentils, peanuts, phaseolus beans and alfalfa sprouts; and in some plants commonly used as flavourings, herbs or spices, e.g. ginseng, fenugreek, sage, quillaja bark, thyme, sarsaparilla and nutmeg (Oakenful and Sidhu, 1990). Their structures are characterized by the presence of a steroid or triterpene group, referred to as the aglycone, linked to one or more sugar molecules. The presence of both polar (sugar) and non-polar (steroid or triterpene) groups provide saponins with strong surface-active properties which then are responsible for many of its adverse and beneficial biological effects(Ambasta, 2000).

A well-known toxic effect of saponin is its ability to lyse erythrocytes, this, in general, being due to its interaction with the cholesterol in the erythrocyte membrane (Birk and Peri, 1980). If provided intravenously to mammals, saponins can cause local inflammation, and in large doses, can result in death due to massive release of erythrocyte debris and reduction in the oxygen-carrying capacity of the blood (Scott *et al.*, 1985). Saponins can also lyse other cells such as those found in the intestinal mucosa and consequently affect nutrient absorption. Decreased weight gain has been observed with high saponin intake due to a number of reasons including reduced food intake attributable to the bitter taste of saponin (Birk and Petri, 1980), or decreased absorption and utilization of nutrients caused either by (a) the inhibition of metabolic and digestive enzymes, e.g. protease, amylase, lipase and cholinesterase inhibition by soya saponin (Cheeke, 1971), and chymotrypsin, protease and succinoxidase inhibition by alfalfa saponins (Birk & Petri, 1980); or (b) binding with nutrients, e.g. Zn binding by alfalfa saponins (West and Greger, 1978).

2.4. Effect of Processing on Nutrient Composition and Anti-Nutritional Factors

Different domestic processing methods (soaking, germination, roasting, boiling, fermentation, and steaming decortications) were used to obtain a suitable texture for the consumer, improvement in the nutritional factors and increase the protein digestibility (Clemente *et al.*, 1998).

2.4.1. Soaking

Traditional treatments such as soaking, cooking, germinating and fermenting have been used to improve nutritional quality of the legume (Kayodé, 2006; Traoré *et al.*, 2004). Soaking usually forms an integral part of processing methods such as germination, fermentation, cooking, dehulling and toasting using different media like water, salts or alkali solutions. Phytate being water soluble, a significant reduction can be realized by discarding the soaked medium (Kumar *et al.*, 2010).

Soaking in water allows the seeds to absorb water, to decrease and eliminate anti nutritional factors in legumes. Soaking is also employed prior to number of other processing treatments such as germination, cooking and fermentation. Soaking also reduces certain ANFs, which leach

into the soaking medium, such as oligosaccharides, protease inhibitors and some tannin (Saxena *et al*., 2003). The amount of leaching will vary depending on the soaking medium (water, salt solution or bicarbonate solution) and soaking time. Soaking also leads to the breakdown several components into simpler compounds which alter the texture, flavour, aroma and taste (Parveen, 2003).

2.4.2. Germination

Food processing technologies can contribute also to the alleviation/improvement of micronutrient deficiencies. Germination is widely used in legumes and cereals to increase their palatability and nutritional value, particularly through the breakdown of certain antinutrients, such as phytate and protease inhibitors (Afify *et al*., 2012).

Germination is a natural process occurred during growth period of seeds in which they meet the minimum condition for growth and development (Sangronis *et al*., 2006). The process starts with the uptake of water by the quiescent/dormant dry seed and terminates with the emergence of the embryonic axis, usually the radical (Bewley and Black, 1994). Several studies on the effect of germination on legumes found that germination can increase protein content and dietary fiber, reduce tannin and phytic acid content and increase mineral bioavailability (Ghavidel and Prakash, 2007).

Germinated seeds have several beneficial properties over ungerminated seeds. Germination improves in vitro protein digestion, as well as fat absorption capacity (Mansour and El-Adawy, 1994) and the extent of germination determines the actual composition. Additionally fenugreek sprouts have shown to be rich in polyphenols, reducing sugars and minerals (K, Zn and Fe) indicating the superiority in the use of germinated seed fractions in functional and nutritional foods compared to their non germinated counter parts (Shakuntala and Naik, 2011). Previous studies carried out by Ghaskadbi *et al.*, 2005 have shown that germinated fenugreek seeds exhibit high antioxidant activity.

Generally, wet processing including soaking, germination and fermentation leads to a reduction in phytic acid and increases of the minerals solubility in foods and could thus improve

bioavailability of minerals in cereals and legumes (Afify *et al.*, 2011). The most effective treatments are fermentation and germination (Elkhalifa and Bernhardt, 2010).

2.4.3. Roasting

Heat treatment significantly improves the protein quality in pulses by destruction or inactivation of heat labile anti-nutritional factors (Wang *et al.*, 2009). Roasting is heat treatment used to induce the development of the typical colour, taste and flavour; it also changes the chemical composition, modifying nutritional value and shelf life (Ozdemir and Devres, 2000).

Roasting causes some desirable or undesirable changes in physical, chemical and nutritional properties of the seeds. One of the desired outcomes of roasting process is the increase in antioxidant activity that occurs due to the formation of Maillard reaction products. The net effect of roasting on the total antioxidant capacity of the seeds depends on the balance between the thermal degradation of naturally occurring antioxidant compounds and the formation of new products having antioxidant activity (Kumaran and Karunakaran, 2007). In food industry, roasting is the process much used to improve the food quality, to extend the shelf-life of foods. The process is carried out for promoting more flavor, desired color and texture changes that ultimately increases the overall palatability.

2.4.4. Boiling

Many processing methods have been shown to reduce antinutrients and improve the nutritive quality of plant foods for human nutrition (Obizoba and Atti, 1994).Boiling and roasting have been reported to enhance taste, flavour and nutritional quality of foods (Sharma and Sehgal, 1992). These treatments may also have adverse effect on some essential nutrients in the foods.

This process improves the appeal and sensory properties of legume. Boiling is usually at 100^0 C for some minutes. It tenderizes the seeds through water absorption. Traditionally, cooking of beans can be done using firewood. Pressure cooking pots allows legumes to be cooked under pressure and it reduces cooking time. This process eliminates heat labile antinutritional factors such as trypsin inhibitors (Bishnoi and Khetarpaul , 1994).

Trypsin and chymotrypsin inhibitors affect the digestibility of legume protein, while other anti-nutritional factors like tannins, phytates, cyanide and hemagglutinins impart bitter or unacceptable taste to the legumes, causing decreased protein digestibility and absorption of divalent metal ions such as F2+ , Zn2+ in the intestine (Abdu *et al*, 2008). Removal of these undesirable components is essential in order to effectively utilize their full potential as feedstuff.

It has been established that cooking and other processing methods exert beneficial effect by destroying the anti-nutritional factors inherent in legume grains (Balogun *et al.*, 2001). Various researchers have boiled or cooked pigeon pea seeds for variable length of time. Boiling generally decreases naturally occurring heat sensitive antinutritive factors and volatile compounds (Akande and Fabiyi, 2010).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Post-Harvest Management laboratory between 2014 and 2015 and Debrezeit agricultural research institute soil and plant pathology laboratory.

3.2. Experimental materials

The *teff* (Kuncho variety) and fenugreek (Challa variety) were used for the purpose of this study. Both varieties were obtained from Debreziet Agricultural Research Center (DZARC) in Ethiopia. It is the first popular high yielding *teff* variety with *teff* productivity of up to 137% from 1.6 tons per hectare to 3.8 tons per hectare (USAID and CIAFS, 2012) and with excellent *injera* making quality.

Figure1 Raw materials used in the experiment.

3.2.1. Preparation of *teff*

The *teff* grain was cleaned manually by winnowing and hand sorting to remove husks, damaged grains, stones, dust, light materials, glumes, stalks, undersized and immature seeds and other extraneous materials followed by milling, (Kaelkolb, D-6072 Dreich, West Germany). Then the flour was sieved (0.5mm) and packed in airtight polythene bag, and stored at room temperature for further processing.

3.2.2. Preparation of fenugreek flour

Fenugreek seeds were cleaned manually by winnowing and hands sorting to remove husks, damaged grains, stones, dust, light materials, glumes, stalks and other extraneous materials then the seeds were packed in airtight polythene plastic bags for further processing**.** As per the experimental treatment, fenugreek seeds were prepared as outlined in Figure 2.

3.2.2.1. Soaking

Seven hundred fifty gram of cleaned fenugreek seeds were soaked in distilled water at a ratio of 1:5 (w/v) at room temperature for 12 h. The water was changed every 6 h. After 12 h, the excess water was discarded, seeds washed with fresh water and seeds were dried with hot air in oven at 40^{o} C for 6 h. The seeds were milled by disk miller (Kaelkolb, D-6072 Dreich, West Germany),sieved (0.5mm) and stored in polyethylene bag for further processing (Hooda and Jood, 2003a).

3.2.2.2. Germination

Fenugreek seeds (750 g) were soaked in potable water of 1:5 w/v for 24 h at room temperature. The excess water was drained and seeds were germinated for 24 hrs at 25^{o}C with the relative humidity of 85 % in the germination cabinet. After 24 hrs, the germinated fenugreek seeds were dried in a drying oven at 40^{0}C for 6 h and milled using disk miller (Kaelkolb, D-6072 Dreich, West Germany), and sieved (0.5mm) to get uniform sized flour (Hooda and Jood, 2004). The flour was packed in airtight polythene plastic bags for further processing.

3.2.2.3. Roasting

Seven hundred fifty gram of cleaned fenugreek seeds were roasted until it become brown. It was continuously stirred with ladle for proper and uniform roasting until it became slight brown and left a peculiar aroma. The seeds were milled by disk miller (Kaelkolb, D-6072 Dreich, West Germany) and sieved (0.5mm)to get uniform sized flour. The flour was then stored in a polyethylene plastic bag for further processing (Pandey and Awasthi, 2012).

3.2.2.4. Boiling

Seven hundred fifty gram of cleaned fenugreek seeds were placed in a beaker with 250 ml boiling distilled water. The beaker is covered with aluminum and the seeds were boiled for 10 min. The boiled seeds were dried in a drying oven at 45^0C for 6h and milled by disk miller (Kaelkolb, D-6072 Dreich, West Germany). The flour was sieved (0.5mm) and kept in polyethylene plastic bag for further processing.

3.3. Experimental design and treatment combination

This study was carried out to find the appropriate concentration of pretreated fenugreek on the nutritional, anti nutritional, mineral composition and antioxidant capacity of *teff**injera*enriched with fenugreek. All the experiments were conducted in a factorial design for ANOVA by combining five fenugreek forms (raw, roasted, soaked, germinated and boiled) and three fenugreek substitution levels (5%, 10% and 15%) and replicated thrice. The list of treatment combination is indicated in Table 3. For each combination, the Physico-chemical, proximate, mineral, anti nutritional composition and antioxidant capacity of *injera* were recorded. For all parameters, data analysis was carried out using SAS version 9.2 software.

Table 3 Details of treatment combinations of pretreated fenugreek and teff flour

No	Pre-treatment methods	Fenugreek (%)	*Teff* (%)
1	Raw(RW) (untreated)	5	95
2	Raw	10	90
3	Raw	15	85
4	Soaked(SD)	5	95
5	Soaked	10	90
6	Soaked	15	85
7	Roasted (RD)	5	95
8	Roasted	10	90
9	Roasted	15	85
10	Germinated (GD)	5	95
11	Germinated	10	90
12	Germinated	15	85
13	Boiled (BD)	5	95
14	Boiled	10	90
15	Boiled	15	85
16	Control(*Teff* only)	0	100

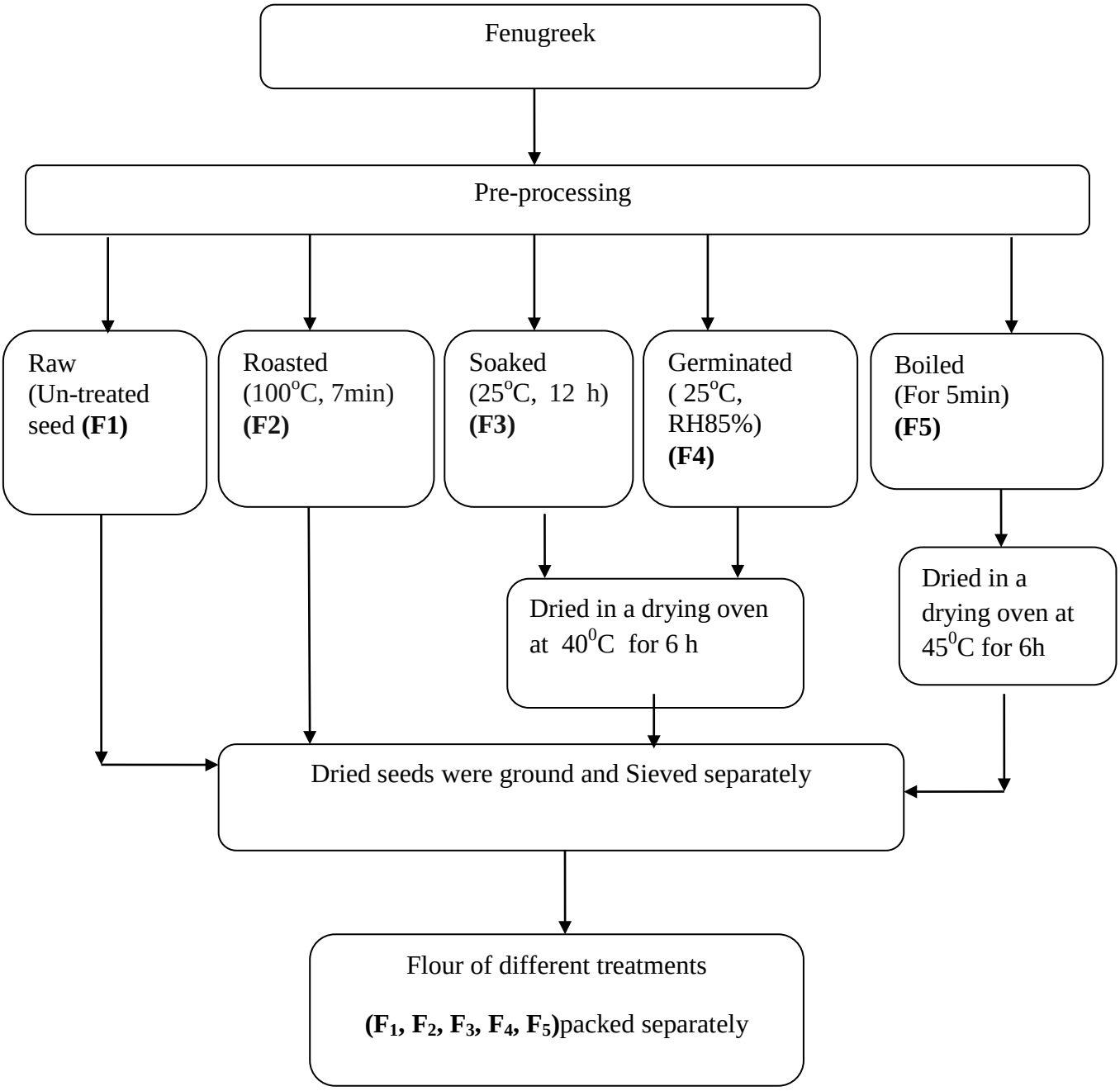

Fig 2 Flow diagram of fenugreek seed flour preparation

3.5. Preparation of blends

Fenugreek seed flour (raw, roasted, soaked, germinated and boiled) was blended separately with *teff* flour at different percentage of 5, 10, and 15% (w/w) as indicated in Table 3.

3.6. Preparation of *Injera*

Teffinjera containing fenugreek seed flour at different concentration was prepared by mixing *teff* and fenugreek (1kg) with clean water in the ratio 1:2 (w/v) and 16 % of starter (*ersho*) by the weight of the flour and was kneaded by hand in a bowl in the traditional.The resultant doughallowed to ferment at room temperature for 72 hr. After this primary fermentation, the surface water formed on the top of the dough was washed and discarded. The dough then was mixed 1:3 (v/v) with boiling water, and heated for 15 min with continuous stirring. After cooling to about 60°C, *absit* (a dough binder) was mixed into fermenting dough for the second phase of fermentation that had lasted for about 2 hr After fermentation, the batter was diluted slightly with water and baked using electrical baking griddle (called *metad*, smoothed surface) covering with a griddle lid (*akambalo*) to prevent steam from escaping. The baking griddle was greased with rapeseed (*Gomenzer*) flour before each *injera* baking to prevent *injera* sticking onto the baking griddle. About half a litter of batter is poured on to the hot clay griddle in a circular motion from the outside and moving towards the centre. After 2-3 minutes of cooking using traditional baking equipment (*metad*), the *injera* was removed and stored in a traditional basket called (*Sefed*).

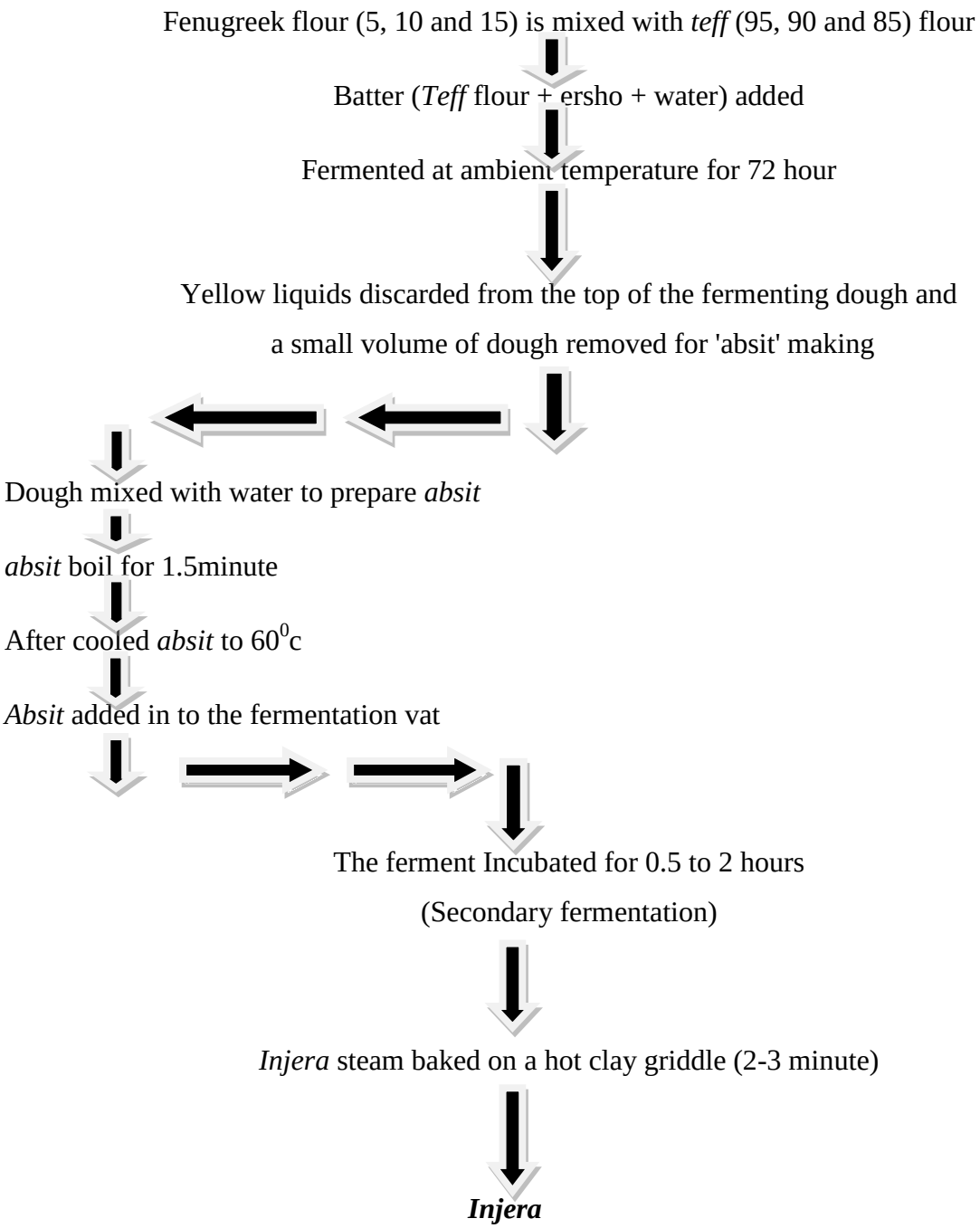

Figure 3 Flow diagram depicting *injera* making procedure (Bemihiretu*et al.*, 2013).

3.7.Data collection

3.7.1.Physical and other chemical property of *injera*

3.7.1.1.Elasticity of *injera*

Tensile strength is the measurement of force to pull *injera*. Good quality *teffinjera* have high elasticity. It was measured by holding and pulling the two ends of *injera.* The change in the tensile strength of *injera* samples from different treatments were measured using the method suggested by Sourki *et al.,* (2010) by using TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK).

3.7.1.2. Thickness of *injera*

Thickness (T) of *injera* was measured with a vanire caliper according to standard methods (A.A.C.C. 2000). The thickness was measured in millimeter with the help of a vanier caliper.

3.7.1.3. Titratable acidity (TA)

In order to determine TA, about 10 g of *injera* was mixed into 100 ml of distilled water followed by filtering through filter paper (Whatman, 1). Then, 10 ml of filtered sample solution was titrated with 0.1N by adding of 3-4 drops phenolphthalein indicator (Akhtar *et al*., 2010). The results were expressed as the percentage of dominant lactic acid in the *teff injera*. At the end, the titratable acidity was calculated using the following formula (Eq1)

$$\%TA = \frac{(N * V1 * Eq.WT)}{(V2 * 10)} \quad \text{.....Eq.1}$$

Where:-

TA= Titratable acidity

N = Normality of titrate (NaOH) (mEq. /ml)

V1 = Volume of titrant used (ml)

Eq.Wt. = Equivalent weight of predominant acid (g)

V2 = Volume the sample (ml)

10= 1/10 is the factor relating mg/g (100/1000)

3.7.1.4. Measurement of pH

The pH value of eachhomogenate *injera* sample was determined using digital pH meter as described by AOAC (2010) official method 981.12. The pH meter was calibrated using pH 4 and 7 buffer solutions. The electrode of the pH meter was then washed with distilled water, blotted with tissue paper and dipped into each of beaker containing liquid samples to measure the pH value.

3.7.2. Proximatecomposition

3.7.2.1. Moisture content

The moisture content was determined as the loss in the weight that results from drying of known weight of food to constant weight using hot air oven (AOAC, 2011) 925.10. The cleaned, dried empty Petridish was weighed (W1). A total of Two grams of each sample was measured in to Petridish (W2). The petridish containing the sample was then placed in oven (Model: Leicester, LE67 5FT, England) at 105^{o}C for 6 hrs, and then cooled in a desiccator and reweighed until the constant mass obtained (W3). Then moisture content was determined using the following equation.

$$\%\, Moisture\ = \frac{W2-W3}{W2-W1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.2$$

Where,

W1= the weight of the petridish,

W2 = the weight of the petridish + the weight of the sample before drying and

W3 = the weight of the petridish + the weight of the sample after drying.

3.7.2.2. Determination of crude protein

The protein content was calculated from the nitrogen content of the *injera*, which was determined by Kjeldahl method involving digestion, distillation, and titration (AOAC, 2005) 988.05. About 0.3 g of sample was measured by analytical balance (model Model:ABJ220-4M, WB1151070, Australia) , one gram of catalyst mixture of K_2SO_4 and $CuSO_4$ and five milliliter of sulfuric acid added to each digestion flask (Kjeldahl flask KF250,German) which contain the

mixture of sample and catalysts. The solution (0.3 g of sample + 1g of K_2SO_4 and copper sulfate + 5ml of H_2SO_4) was immediately placed in digestion flask at about 420^oC for 3-4 hrs, until the solution becomes clear. The digested sample was then transferred into the distillation apparatus and 25ml of 40% (w/v) NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. The mixtures were then steam distilled and the liberated ammonia was collected into a 200ml conical flask containing 25ml of 4% boric acid plus mixed methyl red indicator solution. Next distillation was carried out into the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the pink colour solution of the receiver flask turned green indicating the presence of ammonia. Distillation was continued until the content of the flask was reaching the required amount. The green color solution was then titrated against 0.1N HCl solutions. At the end point, the green colour turned to red pink colour, which indicated that, all the nitrogen trapped as ammonium borate have been removed as ammonium chloride. The distillate was titrated with standardized 0.1N sulfuric acid to a reddish color. Ultimately the percentage of nitrogen content was estimated using the following formula:

$$Nitrogen\,(\%) = \frac{(\ 0.014 \times (Vs - Vb\ ) \times M)}{W} \times 100 \quad \text{.....Eq.3}$$

Where:-

Vs= ml HCl titrant used for test portion

Vb= ml HCl titrant used for blank

M = molarities of HCl solution; and

W = test portion weight, g

Crude protein (%) = 6.26 x %Nitrogen.……..Eq.4

3.7.2.3. Total ash content

The ash content was determined according to AOAC, 2011 method 923. By ignition of a known weight of the food at higher temperature usually at 550^oC in the furnace until all carbon has been removed and the residues appear grayish white. The empty cleaned porcelain crucibles with its lid for the analysis were ignited at 550^oC in furnace (Model: ABJ220-4M, WB1151070, Australia) for 3 hours and cooled in the desiccator. The mass of the crucible was measured (W1)

by analytical balance (Model: ABJ220-4M, WB1151070, Australia). About 3-5g of dried *injera* sample weighed into dried and weighed porcelain crucible (W2). The sample was dried at 120^{o}C for 1hr in drying oven. Then the crucible with its sample was placed in the Muffle furnace and heated at 550˚C for 8 hrs. Finally, the crucible with its sample removed, cooled in the desiccator and weighed (W3). Then weight of ash was calculated as in the following formula (Eq .4):

$$\%Totalash = \left(\frac{W3-W1}{W2-W1}\right) x100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.5$$

Where;

W1 = Weight of empty crucible

W2 = Weight of crucible + sample before ashing

W3 = Weight of crucible + sample after ashing

3.7.2.4. Determination of crude fat

Crude fat content of *injera* sample was determined by Soxhlet extraction according to AOAC, 2005 method, 2003.06. About 1 to 2 g of sample was weighed and put into a thimble. The thimble and its contents were placed in to a 50 mL beaker and dried in an oven for 2 hrs at 102±2^{o}C. The thimble contents were transferred in to extraction unit and extracted with the solvent diethyl ether in a Soxhlet extraction apparatus (SZC-C fat determinate, China) for 1:30 hrs. After the extraction completed, the extraction thimble was dried in an oven for 30 minutes at 102^{o}C ± 2^{o}C to remove moisture. Then it was removed from the oven and cooled in a desiccator. The cup and its contents were weighed. The crude fat was determined by the following formula (Eq.5).

$$Crude\ Fat\ (\%) = \frac{(W2-W1)}{W} X100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.6$$

Where: -

W=Weight of sample

W1= Weight of extraction flask before extraction

W2 = Weight of extraction flask after extraction

3.7.2.5. Determination of Crude Fiber

The crude fiber was determined by the non-enzymatic gravimetric (AOAC, 2011) method, 920.169. Well defatted Two gram food sample were placed into 600 ml beaker and 200 ml of 1.25% H_2SO_4 and 2 g pre weighed boiling chips were added. Then the beaker was placed on digestion apparatus and boiled exactly for 30 min., while shaking at 5 min intervals. The solution was passed through screen sieve and the digested sample was decanted. The digestion beaker was washed with 3 x 50 ml portion of near boiling point water and each transferred into the screen for filtration. The residue left on the screen was transferred into 600 ml digestion flask by washing the screen with 200 ml (50 ml x 4) 1 % NaOH. It was then placed on digestion apparatus and boiled for 30 minute while shaking at 5 min interval. The digested sample was filtered in coarse porosity (75µm) crucible in apparatus at a vacuum of about 25 mm. The residue was dried at 130^{o}C for 2 hrs and cooled in desiccators and weighed (m_1). The dried residue was ignited for 2 hrs at 600±15^{o}C until washing was completed and then cooled in desiccators and reweighed (m_2).

$$Crudefiber\ \% = \frac{M1-M2}{weig\ htofsample}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.7$$

Where,

M_1 = mass of crucible and residue before ignition

M_2 = mass of crucible and residue after ignition

2.7.2.6. Total carbohydrates

Total percentage carbohydrate was determined by the difference method as reported by Ponka *et al.*, 2005 and Onyeike and Oguike (2003). It was determined by subtracting the total percentage values of crude protein, crude fat, moisture, crude fiber and ash constituents of the sample from 100. The value obtained was taken as the percentage carbohydrate constituent of the sample as indicated below (Eq.8).

$$Carbohydrate(\%) = \left[100 - (\%P\ +\ \%Fat\ + \%M + F + \%A)\right] \ldots\ldots\ldots\ldots\ldots\ldots Eq.8$$

Where; P= %protein; F = % fiber; A = % ash and M = % moisture content.

3.7.2.7.Determination of Caloric/Energy Value

Energy value (in Kcal) was determined by multiplying each gram of protein, fat and carbohydrate obtained from laboratory analysis by their respective conversion factor.

$$Caloricvalue\left(\frac{KCa}{100\,g}\right) = (proteinx\ 4) + \ (carbohydratex\ 4) + \ (fatx\ 9) \ldots\ldots\ldots\ldots\ldots Eq.9$$

3.7.3. Mineral analyses (Calcium, Iron and Zinc)

The mineral analyses were determined by Flame Atomic Absorption Spectrophotometer (AAS) (Auto sampler AA 6800, Japan) as per the (AOAC, 2005) method, 985.35. About one gram of composite flour and individual ingredient samples were ashed and weighed. The white ash was treated with 5 mL of 6N HCl and dried on the hot plate. Added 15 mL of 3N HCl and heat the crucible on the hot plate until the solution just boiled. The solution was cooled and filtered through a filter paper in to 50 mL graduated flask and then made up with distilled water. Then the solution was used to determine Ca, Zn and Fe. Standard stock solution of iron, zinc and calcium was made by appropriate dilution. The sample and standard was atomized by using reducing air-acetylene for Ca and oxidizing air-acetylene for zinc and iron as a source of energy for atomization. For iron content determination, absorbance was measured at 248.4 nm and iron was estimated from a standard calibration curve prepared from analytical grade iron nitrate with a range of 0, 2, 4, 6, 8 and 10 mL. For zinc concentration determination, absorbance was measured at 213.9 nm and zinc level was estimated from a standard calibration curve prepared from analytical grade zinc nitrate with a range of 0, 0.5, 1, 1.5, 2 and 2.5 mL. For Calcium content determination, absorbance was measured at 422.7 nm after addition of 2.5 mL of LaCl3 was added to sample solution and standard to suppress interferences. Calcium content was then estimated from standard solution of 0, 2, 4, 6, 8 and 10 mL prepared from CaCO3.

$$mg/100g\ of\ Ca, Fe, Zn = \frac{(Cs - Cb * V)}{(10 * weight\ \ of\ \ sample)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.10$$

Where:-

C_s: Concentration of sample in ppm

C_b: Concentration of blank in ppm

V: Volume (ml) of extract and

W: Weight (g) of dried samples

3.7.4. Determination of anti-nutritional factors

I. Phytate determination

Phytate was determined using the procedure of Latta and Eskin, 1980 and later modified by Vaintraub and Lapteva, 1988. About one gram of dried sample was extracted with 100 ml 2.4 % HCl for an hour at an ambient temperature and centrifuged at 3000 rpm for 30 min. The clear supernatant was collected and 3 ml of sample solution was mixed with 2 ml of wade reagent (0.03% solution of $FeCl3.6H_2O$ containing 0.3% sulfosalicylic acid in water) followed by centrifugation. The absorbance at 500 nm was measured using UV-Vis Spectrophotometer (DU-64 spectrophotometer, Beckman, USA). Absorbance of standard phytic acid solution was measured for the sample after reacting with wade reagent (3mL of water + 2mL of wade reagent). The phytate content of the food sample were estimated from the calibration line and expressed as phytic acid in mg/100g weight of sample.

$$phyticacid = \frac{(Absorbance - Intercept)}{(Slpoe * Density * weight\ of\ sample)} \quad \text{.....Eq.11}$$

Where:

Density= density of HCl

II. Saponin Determination

Saponin content was determined by using procedure of Okwu and Josiah (2006) with some modification. About five gram of the flour samples were mixed with 50mL of 20% aqueous ethanol solution. The mixture was heated in water bath for 90 min at 55^0C. Titration was carried out and the residue collected. The residue was extracted with 50mL of 20% ethanol and both extracts were poured together. The combined extracts were reduced to about 40mL at 90^0C and

transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. The separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partition was done 3 times until the aqueous layer become clear in colour. The saponins were extracted with 60mL of normal butanol. The combined extracts were washed with 5% aqueous NaCl (aq). It was dried at 60^0C in the oven and re-weighed. The experiment was repeated twice and the average taken. The saponin was determined and calculated using the following formula (Eq.12) as a percentage of the original samples.

$$saponin\% = \frac{W2-W1}{W1} X100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.12$$

Where:-

W = weight of sample used

W1 = weight of empty evaporation dish

W2 = weight of dish + saponin extract

3.7.5. Phytic acid/iron, phytic acid/zinc and phytic acid/calcium molar ratios

The contents of phytic acid, iron, zinc and calcium were converted into moles by dividing by their respective molar mass and atomic weight (660.3, 55.8, 65.4 and 40 g mol^{-1}, respectively). The molar ratios of phytic acid/Fe, phytic acid/Zn and phytic acid/Ca were then calculated.

3.7.6. Total antioxidant activity

Preparation of extract: The antioxidant activity of injera prepared from *teff* and fenugreek flours blends were estimated by the method given by Zhang and Hamauzu, 2004. Ten gram of sample was homogenized with 15 mL of 80 % methanol. The homogenate was filtered through filter paper (Whatman,1) and the residue was treated, added with 15 mL of 80 % methanol for 2 successive extractions. The filtrates were combined and centrifuged at 4,000 rpm for 10 min. The supernatant of methanol extract were collected and diluted to various concentrations (1 %, 2.5 %, 5 %, 7.5 % and 10 %) for measurement of antioxidant activity. After the samples at various concentrations were studied, the 10 % concentration was chosen as an appropriate concentration for assessing antioxidant activity.

Free radical scavenging activity-

Antioxidant activity were determined by the 2, 2-diphenylpicryl- hydrazyl (DPPH·method of Brand-Williams *et al.,* 1995. Solutions of DPPH 0.1 mM in methanol were prepared and 4 ml of this solution was treated with 0.2 ml of extract. A control was treated with 0.2 ml of distilled water instead of the extract. The mixture was left to stand for 60 min and absorbance was taken at 517 nm. Total antioxidant activity was expressed as the % of DPPH decrease using the equation:

$$\%Antioxidant\ activity = \frac{Controlabsorbancence\ \ -Sampleabsorbance}{Controlabsorbance} x100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.13$$

3.7.7. Functional property

3.7.7.1. Water absorption capacity (WAC)

Water Absorption Capacity (WAC) was determined using the method of (Adebowale*et al.,* 2005). Ten mL of distilled water was added to 1 g of the sample in a test tube. The suspension was stirred using magnetic stirrer for 3 minutes. The suspension obtained was centrifuged at 3500 rpm for 30 minutes, and the supernatant was measured into a 10 ml graduated cylinder. The water absorbed by the flour was calculated as the difference between the initial volume of the sample and the volume of the supernatant.(Centrifuge model 800-1) for 30 min and the volume of the supernatant was measured in a Ten mL graduated cylinder.

Water absorption =10 ml- final reading from the cylinder

3.7.8. Sensory evaluation

A total of fifty (50) panelists were selected from the staff, undergraduate and graduating class students of Postharvest Management department, Jimma University. Sensory evaluation was conducted within 2 h after baking. The sensory attributes; colour, aroma, taste, texture, appearance and overall acceptability were evaluated using a five point hedonic scale consisting of:

1= extremely dislike)

2= dislike moderately)

3= neither like nor dislike)

4= like moderately)

5 = extremely like) (Muhimbula *et al.*, 2011).

3.7.9. Data Analysis

Factorial experimental design was used for data analysis. Two factor arrangement namely Pre-treatment (5 treatments) and Ratio of fenugreek flour (3) with 3 replications. Total sample was (5x3x3=45). The data were statistically analyzed in a factorial experiment arranged in complete randomized design for ANOVA by employing the generalized linear model (GLM). The collected data from the experiment were analyzed by SAS 9.2 statistical analysis software. For statistically significant results, mean separation was done according to Tukey for $P<0.05$.

4. RESULTS AND DISCUSSION

The influences of pretreated (raw, soaked, germinated, boiled and roasted) Fenugreek seed flour blend with *teff* flour in different proportions on the levels of proximate, antinutrients (phytates and saponins), mineral compositions, antioxidants capacity and sensory property of *injera* were studied and compared with control (100% *Teff**injera*). The results of all the analyzed parameters are presented and discussed under this chapter.

4.1. Proximate Analysis

The proximate composition (moisture, crude protein, ash, crude fat, crude fiber and carbohydrate) of *injera* sample *teff*blended with fenugreek at 5, 10 and 15% levels was determined. The main and interaction effect for all proximate compositions were significantly different ($P<0.05$) except fat and ash.

4.1.1. Moisture content

As indicated in Table 4, the moisture content of *injera* prepared from *teff* enriched with raw, roasted, soaked, germinated and boiled fenugreek flour at 5, 10 and 15% levels ranged from 10.43 to 11.83%. The result showed a significant ($P < 0.05$) on the interaction effects between *Teff* and fenugreek composition. From the obtained data, it was observed that *teff* supplemented with 15% raw fenugreek flour (11.83%) showed the highest moisture content. This might be related to the high moisture content of raw fenugreek as compared to other treated fenugreek blend samples. The results were nearly similar to the findings obtained by Pandey and Awasthi, (2013) the highest moisture content of raw fenugreek than processed fenugreek flour. The next maximum MC was registered from *teff* with 15% boiled fenugreek mixtures (11.61%). This might be due to absorption of water during boiling. Previous findings of Mubarak (2005) reported that boiling of mungbean seeds slightly increased the moisture content of the flour.

Table 4 Mean values of proximate composition of *injera* prepared from blend of *teff* and fenugreek (Dry basis).

Blending Ratio (%)	Proximate composition						
	MC(%)	**C. Protein**(%)	**C. Fat**(%)	**Ash**(%)	**C. Fiber**(%)	**TC**(%)	**Energy(kcal/100g**
Control	10.58^{f}	9.36^{g}	1.91^{i}	2.15^{h}	2.4^{j}	73.49^{a}	348.59^{a}
T:RW							
85:15	11.83^{a}	11.49^{d}	2.73^{a}	3.00^{bc}	3.46^{def}	67.49^{g}	340.49^{fg}
90:10	11.50^{ab}	11.10^{e}	2.60^{ab}	2.96^{bcd}	2.95^{hi}	68.89^{ef}	343.36^{bcd}
95:5	11.30^{abcd}	10.71^{f}	2.38^{bcde}	2.82^{de}	2.65^{j}	70.14^{b}	344.82^{ab}
T:GD							
85:15	11.15^{bcde}	12.95^{a}	2.27^{efgh}	3.19^{a}	3.93^{a}	66.51^{h}	338.25^{h}
90:10	10.70^{ef}	12.27^{b}	2.15^{efgh}	3.10^{ab}	3.87^{ab}	67.91^{fg}	340.40^{fg}
95:5	10.46^{f}	11.78^{cd}	2.05^{h}	2.93^{cd}	3.62^{cd}	69.16^{de}	342.59^{def}
T:RD							
85:15	10.43^{f}	11.78^{cd}	2.28^{defg}	3.02^{bc}	3.57^{cde}	68.92^{e}	342.23^{fg}
90:10	10.58^{f}	11.68^{cd}	2.15^{efgh}	2.92^{cd}	3.15^{gh}	69.04^{cde}	343.23^{cd}
95:5	10.66^{ef}	11.10^{e}	2.11^{fgh}	2.81^{de}	2.95^{hi}	69.96^{cd}	343.38^{bc}
T:BD							
85:15	11.61^{ab}	11.98^{bc}	2.51^{abcd}	2.36^{g}	3.53^{cde}	68.01^{fg}	342.51^{efg}
90:10	11.45^{abc}	11.68^{cd}	2.15^{fgh}	2.32^{g}	3.27^{fg}	69.14^{f}	342.63^{def}
95:5	10.93^{cdef}	11.49^{d}	2.13^{fghi}	2.26^{gh}	3.00^{hi}	69.88^{bc}	344.65^{abc}
T:SD							
85:15	11.38^{abc}	12.75^{a}	2.53^{abc}	2.82^{ed}	3.71^{bc}	66.81^{gh}	341.01^{fg}
90:10	11.28^{bcd}	12.17^{b}	2.33^{cdef}	2.69^{ef}	3.38^{ef}	68.15^{f}	342.25^{efg}
95:5	10.83^{def}	11.58^{d}	2.06^{gh}	2.65^{f}	2.93^{i}	69.9^{b}	344.26^{abc}
SE(±)	0.18	0.10	0.07	0.05	0.07	0.21	0.85
CV (%)	2.93	1.61	5.94	3.45	3.87	0.55	0.43

RW=raw, GD=germinated, RD= roasted, SD= soaked and BD=boiled and MC=Moisture. Means with the same letter (s) are not significantly different ($P<0.05$)

However, the lowest moisture content was observed from *teff* with 15% roasted fenugreek blended *injera* sample (10.43%). This might be due to decrease in moisture content of fenugreek seed flour which can be affected by the roasting temperature and due to shrinkage and lose of moisture. The result is also in agreement with Abayomi *et al.*, (2002) who reported that roasting processes decreased moisture content of peanut.

4.1.2. Crude Protein

In case of protein, the control (100%*Teff injera*) sample showed lower value (9.36 g) in comparison to the injera from blended samples. *Teffinjera*had protein contents 10.72 to 12.95% when blended with fenugreek. The best improvement of protein content of *injera* was obtained from *teff* with treated fenugreek than raw fenugreek blends, though the increment level of protein varied according to the processing methods. The crude protein content of *injera* from blend of *teff* with fenugreek flour was significantly ($p<0.05$) higher than that of control *injera*. This might be due to higher protein content found in fenugreek flour than *teff*. Pandey and Awasthi (2013) reported higher protein content of germinated fenugreek which is (32.7 %).

In the current result highest value (12.95%) was recorded from *injera* prepared from *teff* supplemented with 15% germinated fenugreek flour as indicated in Table 4. The observed increase assumed that due to synthesis of enzyme proteins or a compositional change following the degradation of other constituents Bau *et al.* (1997).

A further explanation was done by Nonogaki *et al.* (2010) where they noted that protein synthesis occurred during imbibition and that hormonal changes play an important role in achieving the completion of germination.The protein content of *injera* was observed to be increasing with increasing blending ratio of the fenugreek flour and also varied among the raw and treated fenugreek seed flour. Similarly, *injera* prepared from *teff* blend with 15% soaked fenugreek showed higher protein content which is about 12.75%. This might be due to water soluble anti-nutrients possibly destroyed during soaking of fenugreek seed. The result is in good agreement with Hooda and Jood (2003b) who reported the highest protein content of bread prepared from wheat and treated fenugreek flour (soaked and germinated).

4.1.3. Ash

Details of the ash content of each *injera* sample are presented in Table 4. The ash content of sixteen *injera* samples showed a significant difference ($P<0.05$) for the main effect and not significant ($P<0.05$) for the interaction effect (Fenugreek type and ratio). The ash content of *injera* was varied from 2.20 to 3.17% for *teff* blended with raw, roasted, soaked, germinated and boiled fenugreek at 5, 10 and 15% levels.

The highest value of ash content from *teff* was noted when supplemented with germinated (3.17%) fenugreek at 15% level. Whereas, the lowest value (2.15%) was recorded from control sample (100% *teff*).These increases have been attributed to dry matter loss, particularly carbohydrates, through respiration, causing an apparent increase in other nutrients such as Ash (Opuku *et al.*, 1981). (Neha and Ramesh, 2012) reported ash content indicates an estimate of the total mineral content in a given quantity of food substance. These finding also agreed with results were reported by Hegazy and Ibrahim (2009) that incorporation of fenugreek flour to biscuits formula increased protein, fat, fiber, ash and amino acids contents.

Generally, among all blendedsamples *teff* with boiled and soaked fenugreek showed minimum ash content. This might be due to leaching out of minerals during soaking and boiling medium. Ukachukwu and Obioha (2000), reported effect of boiling treatment which must have predisposed the seeds to some kind of leaching of some of its mineral elements.

4.1.4. Crude Fat

The crude fat content of *injera* prepared from blend of *teff* with raw, roasted, soaked, germinated and boiled fenugreek seed flour at 5, 10 and 15% ranged from 2.05 to 2.73% as shown in table 4. The fat content of *injera* samples showed a significance difference at ($P<0.05$) for the main effect among all treatments but a non significant difference at ($P<0.05$) for the interaction effect (fenugreek type and ratio) as indicated (Appendix Table 1). The highest value was recorded from *teff* blended with 15% raw fenugreek flour blends (2.73%). This might be due to the high amount of fat present in fenugreek. This result is in agreement with the report of Pandey and Awasthi (2012) who indicated that the higher fat content of raw fenugreek than treated fenugreek flour. But, the lower value was for the control *injera* (1.91%). This might be attributed to the lower fat content of grain *teff* than fenugreek. Girma*et al.* (2013) reported the lower fat content of *injera* prepared from 100% *teff* which is about (2.1%).

As the blending ratio of fenugreek flour increased, so did the fat content of *injera* also increased. All treated fenugreek blend samples showed higher fat value than control but lower than raw fenugreek blend samples. This might be due to the energy consumption during processing of fenugreek as reported by (Kaushik *et al*., 2010). Reduction in fat content upon roasting may be due to loss of volatile oils on open dry heat treatment as reported by (Mathur and Choudhry,

2009). Hooda and Jood (2004) also reported fat content of bread from wheat flour blends with raw fenugreek flour showed the maximum fat content.

4.1.5. Crude fiber

The changes in crude fiber of *injera* from *teff* blend with fenugreek flour sample are presented in Table 4. It showed a significance difference at ($P<0.05$) for *Teff* and fenugreek (type and ratio) interaction (Appendix Table 1). The crude fiber content of *injera* samples which were prepared from *teff* blended with raw, roasted, soaked, germinated and boiled fenugreek flour at 5, 10 and 15% levels was in the range of 2.65 to 3.93%. The higher fiber content was observed in *injera* prepared from *teff* supplemented with 15% germinated fenugreek flour (3.93%). This could be the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination. The increase in crude fiber during germination was reported to be mostly due to changes in the polysaccharides found in the cell wall such as cellulose, glucose and mannose, suggesting that the changes were due to an increase in the cellular structure of the plant during germination (Rumiyati *et al*., 2012).

Hooda and Jood (2003a) reported germination of fenugreek increase crude fiber content. This result indicated that fiber content of *injera* increased with increase the blending ratio of fenugreek flour. On the other hand, the lower value was also recorded from control sample (2.44%). It is known that grain *teff* is a good source of fiber (3.5%) than other cereals since the whole grain is used, however the fiber content of fenugreek (6.0 to 7%) is higher than that of *teff* (Pandey and Awasthi, 2012). This gives an opportunity to boost further the fiber content of *teff* as one of the health food to control the impacts of bad cholesterol.

4.1.6. Total carbohydrate

The total carbohydrate content of *injera* prepared from *teff* blend with fenugreek was shown in Table 4. The values for total carbohydrate varied from 66.51 to 70.14% for *teff* and fenugreek blends (raw, roasted, soaked, germinated and boiled). The carbohydrate content of the control *injera* sample was found to be higher (73.49%) than other *injera* sample prepared from blend of *teff* and fenugreek flour. This might be due to the higher carbohydrate content found in *teff* than fenugreek. The result of this study is comparable with Bultosa and Taylor (2004) who reported a higher carbohydrate content about 74.68% in grain *teff*.

Highly significant (P<0.01) main and interaction effects were observed in respect of total carbohydrate content of 16 *injera* sample. The lowest carbohydrate content (66.51%) was recorded in *teffinjera* blended with 15% germinated fenugreek. The decreased carbohydrate levels of the germinated seeds might be due to increase in α-amylase activity, which breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the of germinated seeds (Inyang and Zakari, 2008). The decrease in the polysaccharide and mucilage content may be attributed to their breakdown and utilization by the growing sprouts (Hooda and Jood, 2003a).This finding indicated that the total carbohydrate content decreased parallel with the increase in the proportion of fenugreek flour. This is because of the low amount of carbohydrate found in fenugreek flour. Hussein *et al*., 2013 indicated that total carbohydrate contents decreased in biscuits fortified with raw, soaked and germinated fenugreek compared to the control (corn flour).

However, as the level of fenugreek flour increased, the carbohydrate content of *injera* decreased. This indicates that *teff* was the main contributor to the carbohydrate content in *injera* (Bultosa, 2007). Generally, the carbohydrate content of all samples which were prepared from blend of *teff* with treated fenugreek showed lower value. This may be due to the increase of other nutrient ratio of the total mass, resulting in redistribution of nutrients percentages and breakdown of carbohydrates.

4.1.7. Gross Energy

Highly significant difference (P<0.01) was observed in the gross energy value among 16 *injera* samples for both main and interaction effects as shown in (Appendix Table 1). The gross energy of *injera* samples prepared from *teff* blended with raw, roasted, soaked, germinated and boiled fenugreek at 5, 10 and 15% levels was varied between 338.25 to 348.59 kcal/100g. The gross energy content of *injera* prepared from *teff* enriched with fenugreek flour was shown in Table 4.

The highest gross energy value (348.59%) was observed in control sample (100%*teff*) and the lowest value was from sample prepared from a blend of 85% *teff* and 15% germinated (338.25%) fenugreek flour. This result indicated that the energy content of *injera* sample prepared from high proportion *teff* flour may be attributed to the high carbohydrate content of *teff* flour. The energy content declined with the fenugreek flour proportion increases in the *teff* flour due to the

low carbohydrate content of fenugreek. The observed decreases in carbohydrate contents in germinated blended samples during germination than other could be attributed to their utilization in the sprouting process as energy sources (Kaushik *et al*., 2010).

4.2. Mineral content (calcium, iron and zinc)

The mineral compositions of *injera* prepared from blend of *teff* and fenugreek flour are given in Table 4. The result indicated that calcium and zinc levels were significantly ($P<0.05$) affected due to ($P<0.05$) the interaction of blends. However, highly significant difference ($P<0.01$) was observed for iron contents on the main and interaction effects (Appendix Table 3).

4.1.8. Calcium

Calcium is the most common mineral in our body and is indispensable for the strength of the skeleton and hardness of teeth (Teegarden, 2003). The present study indicated that calcium content of *injera* prepared from *teff* blended with raw, roasted, soaked, germinated, and boiled fenugreek flour was varied between 79.60 to 103 mg/100g.

The calcium content of *injera* prepared from *teff* blended with fenugreek is significantly ($P<0.05$) different from control *injera* (100% *teff*) for the main and interaction effects as indicated (Appendix Table 3). The highest value of calcium content was obtained from *teff* blended with 15% germinated fenugreek. This result can be correlated with the total ash observed in the germinated samples. The present results are confirmed with Rafik and Laila, 1982 also reported the overall differences in mineral composition of seeds on germination.

The current result also revealed that increased in calcium contents were observed when there was an increase in blending ratio of fenugreek flour in to *teff*. On the other hand, lowest value was for the control (77.28mg/100g) sample. This is due to the fact that fenugreek seed is a good source of calcium than grain *teff*. Birhane (2013) reported that fenugreek seed is rich in micronutrients, calcium, iron and zinc. The result of the current research was also related to other findings Hooda and Jood (2003b) which revealed that supplementation of wheat flour with fenugreek flour significantly increased the total mineral contents.

Table 5 Mean values of mineral composition of *injera* prepared from blend of *teff* and fenugreek (Dry matter basis).

Blending ratio (%)	Mineral composition (mg/100g)		
	Zinc	**Iron**	**Calcium**
Control	2.11^{g}	25.57^{a}	77.28^{f}
T:RW			
85:15	3.08^{cde}	21.42^{cdef}	93.41^{abcd}
90:10	2.99^{cde}	21.83^{bcde}	90.84^{bcd}
95:5	2.70^{defg}	22.64^{bc}	86.47^{def}
T:GD			
85:15	3.93^{a}	21.50^{cde}	103.41^{a}
90:10	3.88^{ab}	21.89^{bcd}	100.23^{ab}
95:5	3.58^{b}	23.09^{b}	97.84^{abc}
T:RD			
85:15	3.52^{b}	21.42^{cdef}	97.68^{abc}
90:10	3.13^{cd}	21.50^{cde}	93.20^{abcd}
95:5	2.85^{def}	22.11^{bcd}	88.74^{cde}
T:BD			
85:15	2.87^{def}	18.64^{h}	87.68^{ef}
90:10	2.70^{defg}	19.78^{gh}	83.15^{def}
95:5	2.52^{defg}	20.47^{efg}	79.60^{ef}
T:SD			
85:15	2.51^{efg}	20.08^{fg}	89.88^{bcde}
90:10	2.37^{fg}	21.24^{def}	87.90^{cdef}
95:5	2.31^{fg}	21.30^{cdef}	83.54^{def}
SE(±)	0.20	0.47	3.70
CV (%)	12.42	3.83	7.13

RW=raw, GD=germinated, RD= roasted, SD= soaked and BD=boiled. Means with the same letter (s) are not significantly different (P<0.05).

4.1.9. Iron

The statistical analysis revealed the presence of highly significant (P<0.01)difference among treatments for iron content. The iron content of *injera* processed from *teff* containing 5% fenugreek was higher than *teff* processed from 10 and 15% fenugreek. This is due to the fact that grain *teff* has naturally high iron contents than fenugreek. The iron content of sixteen *injera* samples prepared from *teff* blend with raw, roasted, soaked, germinated and boiled varied between 18.64 to 23.09 as presented in Table 5. The highest iron (25.57mg/100g) content was for the control (100% grain *teff*) *injera* sample. The iron content of the current result were lower

than 30 to 39 mg/100g that of reported by (Umeta *et al*., 2005). This variation might be partly because of agronomic practices used in *teff* productions and soil type.

Teff blended with 5% germinated fenugreek exhibited the highest value (23.09mg/100g) than all other blends. On the other hand, the lowest value (18.64mg/100g) was obtained from blend of *teff* with 15% boiled fenugreek. This might be due to leaching out of minerals during water boiling. Ukachukwu and Obioha (2000), reported effect of boiling treatment which must have predisposed the seeds to some kind of leaching of some of its mineral elements.This might be due to decrease in phytic acid that increases the availability of minerals. Phytic acid reduces the availability of zinc, manganese, copper, calcium, magnesium, iron as well as protein (Beleia *et al.,* 1993). Hooda and Jood (2003a)also suggested that soaking of fenugreek resulted in a decrease in total Ca, Fe and Zn but germination caused an increase in its contents is in agreement with the current result.

However, there was decrement in the iron content from 25.57 to 18.64 mg/100g with an increase in proportion of fenugreek flour. This is might be due to the higher iron content found in grain *teff* than fenugreek. (Girma*et al.,* 2013) reported that higher amount of iron in *teffinjera*which is about 33.77mg/100g.

4.1.10. Zinc

Zinc content of 16 *injera* samples is presented in Table 5. The result indicated a significant difference ($P<0.05$) observed among all the fenugreek forms and substitution levels. The result of zinc content of the samplesis in a range of 2.1 to 3.93 mg/100g for raw, roasted, soaked, germinated and boiled fenugreek blends. The highest value was obtained from *injera* containing 15% germinated fenugreek (3.93mg/100g). The next highest value obtained from 10% germinated fenugreek (3.88) blended *injera*as compared to other tested samples including control (100% *teff*). There was an increase in zinc content with an increase in the proportion of fenugreek in to *teff*. Nevertheless, the lowest value (2.11mg/100g) was recorded from control sample (100% *teff*). This might be resulted due to the higher zinc content found in fenugreek than *teff*which is about 5.7mg/100g. Similar results were reported by Birhane (2012) where the levels of iron and zinc significantly increase during germination, fermentation, autoclaving, extraction and their combinations. Under this result soaked and boiled fenugreek blended *injera*

samples was showed lowest value than other blends. This might be due to leaching out of minerals by soaking and boiling water. The trend observed was consistent to the results of Hooda and Jood (2003a)who suggested that soaking of fenugreek resulted a decrease in total Ca, Fe and Zn but germination caused an increase in its contents.

4.3. Major anti-nutritional compositions

4.3.1. Phytate

Phytateis commonly found in cereal and legume seeds and its anti-nutritional effect is associated with mineral-complexing (especially Zn, Ca and Fe) and inactivation of digestive enzymes (Frossard *et al.,* 2000). In the current result a significance difference ($p<0.05$) was observed in phytate content among all treatments on the main and interaction effects (Table 6). The highest value was observed from *teff* with 15% of raw fenugreek flour (28.71mg/100g).This might be due to the high amount of phytate found in raw fenugreek flour. The control sample (100%*teff*) exhibited 26.54 mg phytate/100g and it increased significantly with the increase in the level of raw fenugreek flour. The phytate content in the present work is in a range 16.60 to 28.71%.

On the other hand, minimum value (16.60mg/100g) was estimated from *teff* supplemented with 15% germinated fenugreek flour blends. This shows that during sprouting, enzymatic hydrolysis of phytate phosphorus takes place which results in a decrease in phytic acid content. As the blending ratio of treated fenugreek flour increased, the amount of phytate was decreased. The results observed in this study were comparable with (Gupta *et al.,* 2001). Tizazu *et al.,* (2010) also reached at similar conclusion with this result.Similarly, soaked fenugreek blend showed minimum phytate content (18.35%) and a non significant difference was observed with germinated fenugreek blend samples. This might be due to the fact that since phytate is water soluble, considerable amount of phytate might be removed into the water. This result also in agreement with Pandey and Awasthi, 2013 who reported Phytic acid content (552.3–504.2mg /100 g), decreased significantly after soaking.Hegazy and Ibrahim, (2009) and Kumar *et al*., (2010) also reported that soaking process enhances the action of naturally occurring phytase in legumes.

Table 6 Mean values of anti-nutrient composition of *injera* prepared from blend of *teff* and fenugreek (Dry matter basis).

Blending ratio (%)	Anti nutrients	
	Phytate(mg/100g)	**Saponin (%)**
Control	26.54^{bc}	-
T:RW		
85:15	28.71^{a}	0.47^{a}
90:10	27.52^{ab}	0.46^{a}
95:5	24.58^{bcde}	0.43^{ab}
T:GD		
85:15	16.60^{i}	0.34^{def}
90:10	18.35^{hi}	0.31^{fgh}
95:5	22.41^{efg}	0.26^{h}
T:RD		
85:15	20.45^{cdef}	0.41^{abc}
90:10	22.97^{cdef}	0.37^{bcde}
95:5	24.23^{cde}	0.32^{efgh}
T:BD		
85:15	18.98^{hi}	0.42^{abc}
90:10	20.59^{gh}	0.39^{bcd}
95:5	22.55^{defg}	0.32^{efg}
T:SD		
85:15	19.12^{h}	0.39^{bcd}
90:10	22.82^{fg}	0.36^{cdef}
95:5	23.04^{def}	0.26^{gh}
SE(±)	0.70	0.35
CV (%)	6.56	10.60

RW=raw, GD=germinated, RD= roasted, SD= soaked and BD=boiled. Means with the same letter (s) are not significantly different ($P<0.05$).

4.3.2. Saponin

Saponins are secondary compounds that are generally known as non-volatile, surface active compounds which are widely distributed in nature, occurring primarily in the plant kingdom (Shanthakumari *et al.,* 2008). The data regarding saponin content of *teff* supplemented with fenugreek flours is given in Table 6. The result shows a highly significant difference ($p<0.01$) among samples in the main and interaction effects. The saponin content of *injera* made from blend of *teff* and fenugreek flours was in the range of 0.43 to 0.47, 0.32 to 0.41, 0.26 to 0.39, 0.26 to 0.34 and 0.32 to 0.390% for raw, roasted, soaked, germinated and boiled respectively. The highest value was observed from *teff* blended with 15% raw fenugreek which is about

(0.47%). The saponin content of the sample containing 10% and 5% raw fenugreek blend shows higher value than other treated fenugreek blend samples and a non significant difference with 15% blend samples. This might be due to the highest amount of saponin in raw fenugreek seed.

The saponin content increased as the ratio of raw fenugreek flour increased in the *teff* flour. The level of saponin observed in this study was comparable to Birhane, 2012 who reported the highest amount of saponin in raw fenugreek. However, the lowest value was recorded from *teff* supplemented with 5% germinated fenugreek flour (0.26%). Similarly *teff* blend with 5% soaked seed (0.27%) showed the lowest value as compared to other processed fenugreek supplemented *injera*. In this result saponin content was found to decrease due to leaching out of saponin in water. Similar result reported by Birhane, (2012) saponins during germination is attributed to the solubility of saponins in water.

4.4. Molar ratios and bioavailability of minerals

The molar ratio of phytate/mineral of all *injera* samples analyzed are summarized and shown in Table 7. The molar ratios of [phy]: [Zn] of all *injera* samples were <5 indicating a good zinc bioavailability. Similarly the molar ratio of [phy]: [Ca] of <0.24 indicating good calcium bioavailability also. For iron content, all *injera* samples had good bioavailability with [Phytate]: [Fe] molar ratio <1.

4.4.1. Phytate:Calcium

Phytic acids markedly decrease Ca bioavailability and the Ca:Phy molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of [phy]: [Ca] of < 0.24 indicating good calcium bioavailability (Woldegiorgis *et al.*, 2014). The values in the present study were lower in all *injera* samples than the reported critical molar ratio of Phytate to Calcium as indicated in tables 7, indicating that absorption of calcium not adversely affected by phytate in all pretreated fenugreek blended samples. The molar ratios of Ca: Phy in raw, roasted, soaked, germinated and boiled fenugreek blended *injera* samples was in theranged of 0.008 to 0.021mol/kg.

All preteated fenugreek blended *injera* samples analyzed in this study exhibited phytate: calcium molar ratios less than 0.24 mol/kg, however germinated fenugreek blended *injera* sample shows

low value of Phytate to Calcium molar ratio (0.008 mol/kg) which indicated that calcium is available for absorption from *injera* samples (good bioavailability) than other samples. This result is similar with Hooda and Jood (2004) who reported blends containing germinated fenugreek flour showed higher percent availability of Ca, Fe, and Zn compared to raw fenugreek supplemented blends.

Table 7 Calculated molar ratio Phy: Fe, Phy: Zn and Phy: Ca molar ratios of injera from teff and fenugreek blends(mol/kg)

Pre-treatment types	Phytate:Zn	Phytate:Fe	Phytate:Ca	Phytate*Ca: Zn
Control(100% teff)	1.24	0.088	0.021	2.09
T:RW				
85:15	0.925	0.113	0.019	2.13
90:10	0.911	0.107	0.018	2.09
95:5	0.907	0.092	0.017	1.94
T:GD				
85:15	0.418	0.065	0.008	1.07
90:10	0.468	0.071	0.011	1.16
95:5	0.620	0.082	0.013	1.53
T:RD				
85:15	0.575	0.081	0.012	1.40
90:10	0.727	0.091	0.014	1.69
95:5	0.843	0.093	0.016	1.85
T:BD				
85:15	0.656	0.086	0.012	1.43
90:10	0.756	0.088	0.015	1.56
95:5	0.887	0.093	0.017	1.78
T:SD				
85:15	0.756	0.081	0.012	1.65
90:10	0.960	0.091	0.015	2.06
95:5	0.996	0.091	0.016	2.09

4.4.2. Phytate: Iron

Phytate begins to lose its inhibitory effect on iron absorption when phytate:iron molar ratios are less than 1.0, although even ratios as low as 0.2 exert some negative effect (Hurrell, *et al.*, 2003). The phytate:iron molar ratios greater than 0.15 regarded as indicative of poor iron bioavailability (Siegenberg *et al.*, 1991). The result in table 7 indicated that phytate:iron molar ratios of raw, roasted, soaked, germinated and boiled fenugreek blended teff *injera* was in the range of 0.065 to

0.113 mol/kg. The values in the present study were lower than the reported critical molar ratio of Phy: Fe, indicating that absorption of Fe not adversely affected by phytate in these teff-fenugreek blended *injera.*

The phytate: iron molar ratios of teff blended with pretreated fenugreek shows low value of phytate: Fe molar ratio which is below the critical value <1 which implies the absorption of iron all the accessions not inhibited by phytate and as a result the bioavailability of iron is good. Among all *injera* prepared from teff blended with germinated fenugreek was shows lower phytate: iron molar ratio below the critical value (0.065 mol/kg) which indicates of good bioavailability. When the phytate: iron molar ratio > 1 indicates low iron bioavailability (Tizazu *et al.*, 2010). The present results are confirmed by Hooda and Jood (2003) who demonstrated that soaking and germination improved the availability of Ca, Fe and Zn in fenugreek as compared with raw. Another study is in agreement with the current finding ,they suggested that all the processing subjected to mungbean (soaking, germination, cooking, fermentation and dehulling) resulted in an increase in iron bioavailability in vitro; the maximum bioavailability was in germinated cooked mungbean, followed by fermented cooked mungbean and germinated raw mungbean (Barakoti and Bains, 2007).

However, processing of fenugreek seed resulted in a reduction of the phytate: iron molar ratios, especially during germination. In general, phytate mineral ratio was decreased significantly after each processing methods (Table 7). The lower phytate: mineral ratios from the processed fenugreek blended samples may be partly attributed to the decreased in content of phytic acid during the treatments which has a significant negative correlation ($p< 0.05$) with the phytate: mineral ratio (bioavailability of minerals).

4.4.3. Phytate:Zinc

Phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH (Oberleas, 1983). The formation of the chelates depends on relative levels of both zinc and phytic acid (Davies, 1979). Phytate: zinc molar ratio is used to estimate the likely absorption of zinc from a diet. Tizazu *et al*., (2010) elucidated that diet with a phytate: zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with phytate: zinc

molar ratios between 5 and 15 have medium zinc bioavailability and those with a phytate: zinc molar ratio less than 5 have relatively good zinc bio-availability.

The results in tables 7 indicated low values (phytate: zinc molar ratio <5) were found in all *injera* samples prepared from blend of teff with fenugreek flour including control (100%teff), however, *injera* made from blend of teff and pretreated fenugreek flour shows low value of phytate: Zn molar ratio as compared to control *injera* sample (100 teff%), indicative of favorable zinc bio-availability which is less than thecritical value 15. This means that Zn obtained from all *injera* samples would be bioavailable for the human body. The phytate: zinc molar ratios were varied between 0.418 to 1.24mol/kg. Among all tested samples germinated fenugreek blended *injera* sample shows lower value of phytate to zinc molar ratio (0.418 mol/kg) which is an indication of good bioavailability. The increase in bioavailability (low Phytate:Zn molar ratio) of minerals is due decrease in phytic acid which binds minerals. The reduction of phytic acid during germination is due to an increase in the phytase activity, which degrades phytic acid in plant based foods.

Generally, The lower phytate: mineral ratios from the processed fenugreek blended *injera* samples may be partly attributed to the decreased in content of phytic acid during the treatments. Khetarpaul and Chaufan (1989) observed germination of pearl millet for 24 hr resulted in a significant improvement of availability of Ca, Fe, Zn, Cu and Mn.

4.5. Total antioxidant Capacity

The antioxidant capacities of the current results were presented in figure 4. The result of antioxidant property of *injera* prepared from blend of *teff* and fenugreek extracts were studied by its ability to reduce the DPPH. The DPPH test indirect method for determining the antioxidant activity, which is based on the ability of the stable free radical 2, 2-diphenyl-1- picrylhydrazyl to react with hydrogen donors including phenols (Roginsky and Lissi, 2005).The decrease in absorbance of the DPPH radical caused by antioxidant was due to scavenging of the radicals by hydrogen donation is visually noticeable as a color change from purple to yellow.

Results of the activity of free radical scavenging of the 16 samples extracts are presented in Figure 4. Results showed significant difference ($P<0.05$)among all treatment for the main and

interaction (pre treatment and concentration) effects. *Injera* which were prepared from *teff* blended with pretreated fenugreek flour varied from 32.53 to 57.69%.

Among all samples, *teff* blended with 15% germinated fenugreek flour showed the highest antioxidant activity of about 57.69%. Similarly, *teff* with 15% soaked fenugreek extract also showed the highest antioxidant activity of about 56.5%.These increases could be due to the biosynthesis and bio accumulation of phenolic compounds as a defensive mechanism to survive under environmental stresses, like cold exposure (Randhir *et al.,* 2004), and to degradation of polymerized polyphenols, specifically hydrolysable tannins, and the hydrolysis of other glycosylated flavonoids (Monagas *et al.,* 2005). The highest antioxidant activity of samples may be the presence of flavonoids in germinated fenugreek seeds as reported by (Dixit *et al.*, 2005). Shakuntala *et al.,* (2011) also reported that sprouts of germinated fenugreek seeds were rich in polyphenols (97.55 mg/100 g).

On the other hand, the minimum antioxidant activity of 32.53% was found in control sample (100% *teff*). This is due to the antioxidant capacity of fenugreek which is higher than that of *teff*. Bemihiretu*et al.,* (2013) reported the IC50 of *injera* enriched with fenugreek partly fermented white *teff* showed higher scavenger property (2.63 mg/ml) than *teff* alone. Generally, the results of this study indicated that the antioxidant contents of *injera* prepared from *teff* blended with raw and pre processed fenugreek had different antioxidant capacity. All pre-processed fenugreek blended samples showed better antioxidant capacity than the raw fenugreek blend and control (100%) *injera* samples. This might be due to the processing method improve the antioxidant capacity of fenugreek.

From the figure what is observed in all cases at higher concentration there was a higher antioxidant capacity and other pre-treatment conditions like germination, soaking and boiling further increases antioxidant capacity of blends.

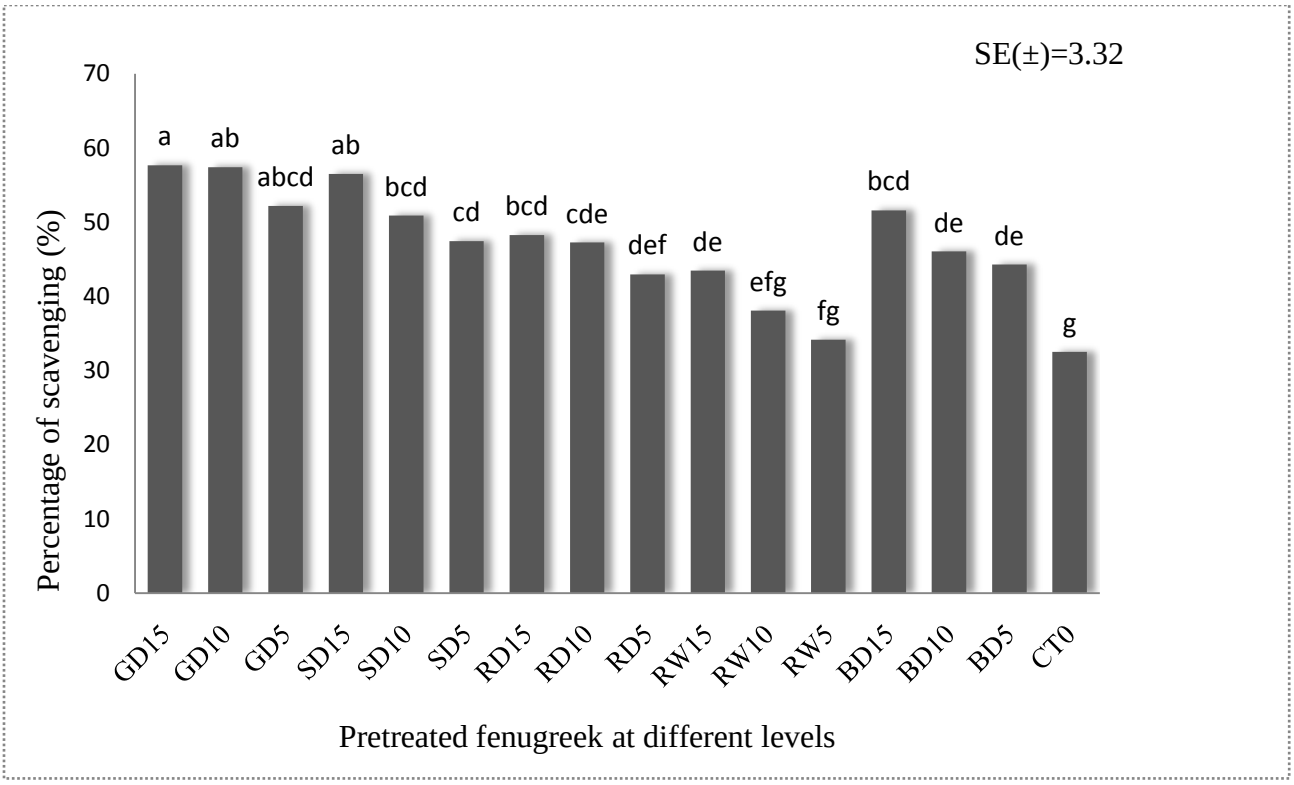

Figure 4 Total antioxidant activities of *injera* samples which were prepared from *teff* and fenugreek.Bars with the same letter (s) are not significantly different (P<0.05)

4.6. Physical property of *injera*

4.6.1. Elasticity (stretchiness) of *injera*

The elasticity of *injera* samples made from *teff* and fenugreek blends was performed using a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK). The *injera* elasticity which were prepared from *teff* blend with fenugreek flour showed a highly significant difference (P<0.01) among all samples in the main and interaction effects as indicated (Appendix table 2) The range of tensile strength of different injera samples was between91.83g to 117.46g.

Sample containing 15% germinated fenugreek flour shows greater elasticity (need greater force) values compared to the rest samples those containing 5 and 10% fenugreek. Whereas the lowest 91.83gelasticity strength was recorded for *injera* with 100% *teff*. The increment could be attributed to the higher expansion of *injera* samples containing fenugreek due to the increased levels of available protein. This increased in elasticity was directly proportional to the amount of fenugreek flour added and these differences were greater at 15% inclusion. Similarly, Malomo,

2012 reported bread substituted with from flour of germinated soybeans showed greater hardness than those substituted with flour from non- germinated soybeans.

4.6.2. Thickness

During baking, the batter was manually poured and spread on the hot clay griddle *(mitad).* Thus, the thickness of the *injera* depended on the consistency of the operator. Theelectrically heated *mitad* used for baking was not thermostatically controlled. Hence, thetime which elapsed between applications of batter on the hot clay griddle needed to bekept as constant as possible to limit variations in batter cooking temperature.The result of the current study was shown in Table 6. Thickness of *Injera* samples which were prepared from blend of *teff* with raw, roasted, soaked, germinated and boiled fenugreek flour were varied from 2.83 to 4.66 mm. As the result of this study, fenugreek flour had a significant difference at ($P<0.05$) among all samples in the main effect and insignificant in the interaction effect ($P<0.05$). The data indicated that the highest thickness was obtained from 5% fenugreek blend and none significant difference was observed from the control sample. But, the lowest was for the sample with raw fenugreek at 15% level. However, almost the thickness of all *injera* samples containing 15% fenugreek flour was not significantly different and showed lower value. Generally, decrease in thickness of *injera* with increasing fenugreek flour in *teff* blends was observed. The thickness of injera is dependent on the amount of water added in to dough, the fermentation process and distribution of dough during baking.

4.7. Other chemical property

4.7.1. Titratable Acidity and pH

The TA content was high (low pH) for fenugreek containing *injera* and increased (decreased pH) as the fenugreek supplementation increased ($P < 0.05$). The titratable acidity of *injera* samples was found to have inverse relationship with pH. As pH decreased in *teff*-fenugreek blend samples, a significant ($P<0.05$) increase in titratable acidity was found. It increased from 0.1 (control) to 0.37 (15% germinated fenugreek blend sample) The pH of *injera*samples prepared from *teff* supplemented with pretreated fenugreek was in the range of 3.51 to 3.87as indicated in Table 7.

The highest TA (0.37%) (Low pH 3.51) was for *injera* processed from *teff* blend with 15% germinated fenugreek. While, control sample (0.10% TA, high 4.40 pH) had the lowest than other tested samples. The pH found for control *injera* was in close agreement with Girma (2012) which is about 4.49%. The increase in TA in germinated fenugreek blend sample might be due to that during germination starch is hydrolyzed into sugars which is readily utilized by the organisms and converted to lactic acid (Jood *et al.*, 2012). Decrease in pH and an increase in TA as the ratio of fenugreek increased in the *teffinjera* because fenugreek were also undergoing fermentation in part producing more acids and similar actions might be happened in this work.

Table 8 Mean values of physical and other chemical property of *injera* prepared from blend of *teff* and fenugreek (Dry matter basis).

Blending ratio (%)	Sensory evaluation			
	Elasticity(g)	**Thickness(mm)**	**TA**	**pH**
Control	91.46^{f}	4.16^{abc}	0.1^{h}	4.40^{a}
T:RW				
85:15	97.16^{cdef}	3.00^{e}	0.21^{efg}	3.71^{b}
90:10	94.00^{def}	3.33^{de}	0.19^{fg}	3.74^{b}
95:5	91.83^{ef}	4.03^{bc}	0.18^{g}	3.87^{ab}
T:GD				
85:15	117.46^{a}	3.00^{e}	0.37^{a}	3.51^{j}
90:10	105.86^{abcd}	4.33^{ab}	0.31^{abc}	3.60^{hi}
95:5	102.43^{bcdef}	4.33^{ab}	0.25^{cdefg}	3.63^{fgh}
T:RD				
85:15	109.83^{abc}	3.30^{de}	0.28^{bcde}	3.62^{fgh}
90:10	102.46^{bcdef}	4.23^{abc}	0.24^{cdefg}	3.67^{efg}
95:5	99.46d^{bcdef}	4.66^{a}	0.23^{defg}	3.68^{cd}
T:BD				
85:15	109.40^{abc}	3.00^{e}	0.29^{bcd}	3.57^{i}
90:10	104.43^{abcdef}	3.66^{cd}	0.26^{cdef}	3.67^{efg}
95:5	99.76^{bcdef}	4.00^{bc}	0.23^{defg}	3.68^{cd}
T:SD				
85:15	113.00^{ab}	2.83^{e}	0.34^{ab}	3.61^{gh}
90:10	105.60^{abcde}	4.53^{ab}	0.27^{cde}	3.65^{def}
95:5	99.30^{bcdef}	4.53^{ab}	0.24^{cdefg}	3.68^{cde}
SE(±)	3.07	0.21	0.04	0.02
CV (%)	4.48	9.83	16.61	0.75

RW=raw, GD=germinated, RD= roasted, SD= soaked and BD=boiled. Means with the same letter (s) are not significantly different (P<0.05)

4.8. Functional property

4.8.1. Water absorption capacity

Water absorption characteristics represent the ability of a product to associate with water under conditions where water is limiting (Singh, 2001).The water absorption capacity (WAC) of samples prepared from blend of *teff* and fenugreek flours are shown in (Figure 5) WAC of *teff* blend with raw, roasted, soaked, germinated and boiled fenugreek flour at 5, 10 and 15% levels was varied between 3.27 to 3.51 ml respectively. The WAC of the present study showed highly

significant difference (P<0.01) among all samples in the main effect and a non significant difference was observed in the interaction effects (Appendix Table 2).

The WAC showed decreasing trend as the level of fenugreek flour increased in the blends. This result may be observed due to the low carbohydrate content of fenugreek flour and processing effect. Basically, processing decreases the starch content of seeds. As indicated by Lawal and Adebowale (2004) chemical composition that enhances the WAC of flours is carbohydrates; since this component contain hydrophilic parts, such as polar or charged side chains. According to Kaur and Sing (2005)**,** flours with high water absorption have more hydrophilic constituents, such as polysaccharides. Therefore, the higher water absorption capacity of 100% *teff* flour (3.67mL) than the other blend of *teff* with fenugreek flours could be attributed to the presence of greater amounts of hydrophilic constituents. *Teff* flour has high water absorption capacity, which relates to the higher degree of swelling of the *Teff* starches, which have a small and uniform granule size, hence, providing larger surface area and thus higher water absorption (Bultosa 2007).The lower water absorption capacity (3.27%) was for the *teff* flour containing 15% germinated fenugreek flour. This might be due to the water holding capacities of starch granule which was affected by processing; particularly germination.

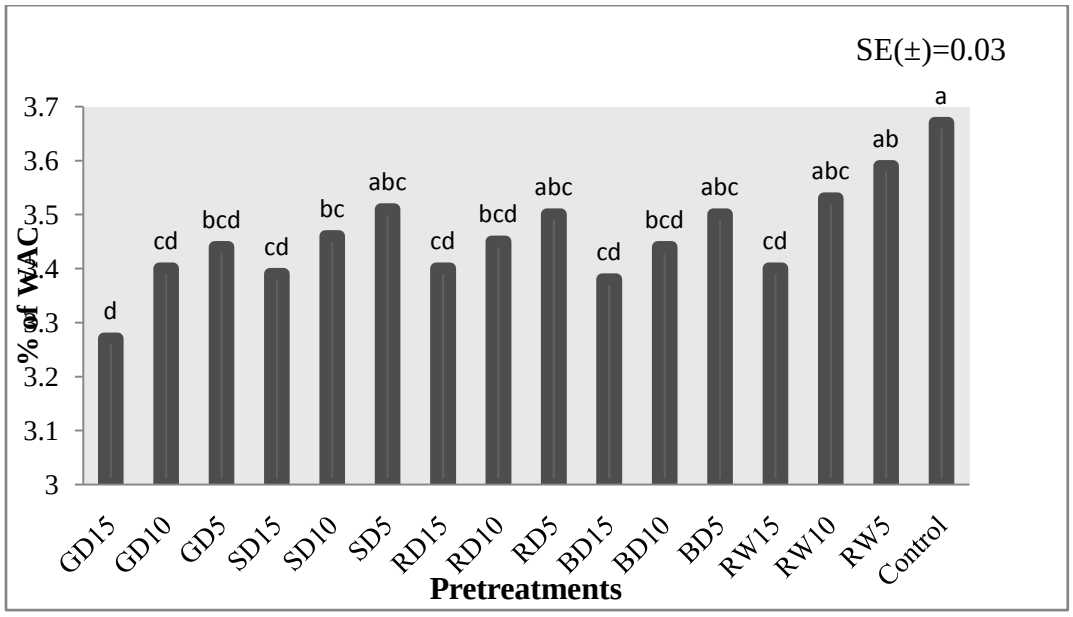

Figure 5 Water absorption capacity of *teff* and fenugreek blended flour.
Bars with the same letter (s) are not significantly different (P<0.05)

4.9. Sensory Evaluation

Sensory qualities are the main criterion that makes the product to be liked or disliked (Falola *et al., 2011*). Descriptive sensory attributes of mean scores given by the sensory panel for colour, aroma, taste, texture, appearance and overall acceptability are presented in Table 8.

Analysis of variance showed a significant difference ($P<0.05$) between *injera* made from *teff* flour (control) and blend with fenugreek flour at 5, 10, and 15% in terms of colour, flavour, taste, texture, appearance and overall acceptability. *Teff* with 15% raw fenugreek were judged with lowest score for most of sensory evaluation parameters and the other blends of *teff* with 15% pretreated fenugreek *injera* were at an acceptable limit, but their scores given by the panelists were lower than other blends.

Injera supplemented by raw, roasted, soaked, germinated and boiled fenugreek flour at 5, 10 and 15% were sensory evaluated and compared with control *injera* (100% *teff* flour) as shown in Table 8. Data indicated that there were no significance difference among control *injera* and *injera* samples containing 5% fenugreek seed flour in all sensory characteristics. However, *Injera* containing 10% and 15% fenugreek flour were showed significantly different ($P<0.05$) from control sample and samples supplemented with 5% fenugreek flour in all properties.

Table 9 Mean values of sensory property of *injera* prepared from blend of *teff* and fenugreek.

Blending ratio (%)	Colour	Aroma	Taste	Texture	Appearance	Overall Acceptability
Control(100:0)	4.34^{a}	4.0^{ab}	4.32^{a}	3.92^{abc}	4.02^{ab}	4.46^{a}
T:RW						
85:15	2.57^{ef}	2.77^{d}	2.28^{f}	2.58^{g}	2.0^{f}	2.10^{h}
90:10	3.26^{ed}	3.14^{bcd}	2.93^{de}	2.82^{fg}	3.10^{cd}	3.21^{fg}
95:5	3.76^{ab}	3.38^{bc}	3.10^{bcde}	3.14^{def}	3.12^{bcd}	3.30^{cdef}
T:GD						
85:15	2.60^{ef}	3.10^{bcd}	2.83^{ef}	$3.24d^{cdef}$	3.04^{cd}	3.00^{ef}
90:10	3.43^{abc}	3.37^{bcd}	3.45^{abcd}	3.62^{bcd}	3.33^{bcd}	3.43^{bcdef}
95:5	3.72^{abc}	3.47^{b}	3.85^{ab}	4.20^{a}	4.08^{a}	4.10^{ab}
T:RD						
85:15	2.55^{ef}	2.83^{cd}	2.95^{de}	3.34^{cdef}	2.95^{de}	2.87^{fg}
90:10	3.52^{bcd}	3.55^{bcd}	3.16^{bcde}	3.70^{bcde}	3.59^{abcd}	3.51^{abcde}
95:5	3.64^{ab}	4.10^{a}	3.55^{abcd}	3.50^{cde}	3.61^{abc}	4.00^{abc}
T:BD						
85:15	2.05^{f}	2.87^{cd}	2.65^{ef}	2.98^{efg}	2.41^{ef}	2.34^{gh}
90:10	3.01^{cde}	3.20^{bcd}	3.07^{cde}	4.00^{ab}	3.03^{cd}	3.14^{ef}
95:5	3.74^{ab}	3.61^{abc}	3.52^{abcd}	3.64^{bcd}	3.72^{abc}	3.80^{abc}
T:SD						
85:15	2.95^{cde}	2.79^{cd}	2.63^{ef}	3.28^{cdef}	3.06^{cd}	3.18^{def}
90:10	3.24^{bcd}	3.22^{bcd}	3.20^{bcde}	3.74^{bcd}	3.26^{bcd}	3.57^{abcde}
95:5	3.75^{ab}	3.59^{abc}	3.67^{bc}	3.46^{cde}	3.63^{abc}	3.73^{abcd}

RW=raw, GD=germinated, RD= roasted, SD= soaked and BD=boiled

Means with the same letter (s) are not significantly different ($P<0.05$)

4.9.1. Color

Vision plays a major role in sensory analysis and the appearance of food can have a major effect on its acceptability (Kikafunda *et al.,* 2006). The color of *injera* prepared from blend of *teff* with raw, roasted, soaked, germinated and boiled fenugreek flour at 5, 10 and 15% levels showed a significant difference ($P<0.05$). The *injera* samples prepared from 100% *teff* were the most preferred than others with the mean value of (4.34) and most of 5% fenugreek blended*injera* samples were noticed at par with control *injera*. Normally, white *injera* colour is preferred by consumers. The whiteness was reduced as the fenugreek substitution levels increased. Similar observation was reported by Sharma and Chauhan (2000) where decrease in color intensity with the increase in the level of substitution of fenugreek flour in wheat bread.

4.9.2. Aroma

The Aroma of food is very important for its acceptability and a slight change in the odour of processed food may affect the overall quality of the product. The Aroma of the *injera* samples showed significant difference ($P<0.05$) in the main and non significance on the interaction effects. Control *injera* samples rated significantly at par with *injera* samples made from 5% supplemented roasted fenugreek flour. The sensory evaluation revealed *Injera* samples supplemented with fenugreek flour up to 10% levels are rated within acceptable limit by sensory panels but noticed lower acceptance for 15% supplementation level with the mean score of below3. According to the performance of the panelists, the majority gave the sensory preference for the aroma to the sample of *teff* supplemented with 5% roasted fenugreek (4.10) and followed by for the control (4) respectively.

4.9.3. Taste

Taste is an important sensory attribute of any food. The mean value of taste was found to be in the range of 2.28 to 3.67 (Table 8). A significant difference ($P<0.05$) was observed in the interaction effect. The highest mean score for taste was obtained from *teff* (95%) and fenugreek (5%) blend *injera* samples. From all tested samples, 100% *teffinjera* showed the highest score (4.32) followed by 5% germinated fenugreek blended sample (3.85). However, among all, 15% fenugreek blended samples were scored the lowest mean (below 3) evaluated by majority of panelists. This might be due to the bitter taste of fenugreek flour. Addition of high proportions of the fenugreek in to *teff* may introduce objectionable characteristics which overwhelmed the traditional taste attribute of the pure *teffinjera* and affected the choice of their taste. The acceptability of the taste was increased with the level of fenugreek proportion decreased due the bitter taste of the seed.

4.9.4. Texture

Texture fundamentally was important in determining the consumer acceptability of baked products.Textural sensory characteristics of 16 *injera* samples including control were presented in Table 8. The mean texture value of *injera* samples prepared from raw, roasted, soaked, geminated and boiled at 5, 10 and 15% levels was ranged from 2.58 to 4.20. A highly significance difference ($P<0.01$) was observed among the treatments at 15% fenugreek blend for

the main effect and a non significant difference for the interaction effect. The average mean value of texture was showed the highest score at 5% germinated in all fenugreek flour blends as compared to 10 and 15% blends. But, the lowest score was for *injera* prepared from 85% *teff* with 15% raw fenugreek flour blends. Generally, the texture of *injera* from blend of 95% *teff* with 5% fenugreek flour showed highest score and at par with control *injera* samples and 10:90 are within acceptable limits.

4.9.5. Appearance

Appearance is an important attribute in food choice and acceptance. Outcome of sensory evaluation indicated that some samples were similar in appearance while others differed significantly. Appearance of *injera* which were prepared from blend of *teff* with fenugreek (raw, roasted, soaked, germinated and boiled) at 5, 10 and 15% levels was in the range of 2.0 to 4.08. Panelists rated blend of *teff* with 5% germinated and control samples significantly higher ($P < 0.05$) than the rest of evaluated samples. *Injera* samples which were prepared from blend of *teff* with 10% fenugreek had values within acceptable limit.

Generally, *injera* prepared from 100% *teff* and supplemented with 5% fenugreek flour were found to be more appealing and liked by majority of the panelists. Samples from *teff* with 15% fenugreekscored poorly in terms of appearance and were below average with mean scores of 3 given by majority of panelist.

4.9.6. Overall acceptability

The result showed that overall acceptability score of all the *teff* supplemented with fenugreek *injera* at 5% level was found nearest to control. *Injera* made from *teff* and raw, roasted, soaked, germinated and boiled fenugreek flour at 5, 10 and 15% levels showed a highly significance difference ($p<0.01$). The result was in the range of 2.10 to 4.46.

Among all 16 samples, *injera* prepared from 100% *teff* was the most accepted with the mean score value of 4.46 in overall acceptability than the other *injera* samples.This might be due to experience and taste of the panelists for the control sample as compared to others.Nevertheless, *injera* samples prepared from 85% *teff* and 15 % raw fenugreek were rejected as compared to the others with a mean value of below 3. This result implied that, when fenugreek flour increased,

the acceptability of the product decreased. This is because of the bitter taste of fenugreek flour due to the presence of saponin in the seeds (Birhane, 2013). Overall acceptability of *injera* samples which was made from blend with pretreated fenugreek up to 10% was within acceptable limits. The current result is in agreement with Hooda and Jood (2004) who reported fenugreek flour in wheat flour was found acceptable up to the levels of 10% in biscuits.

5. SUMMARY AND CONCLUSION

Injera is the major staple food for the majority of Ethiopian and as well as in developed world. *Teff* is known to have better nutritional value than common cereal grains (wheat, barley, sorghum, maize and rice) because grain *teff* is always consumed as whole grain but, it is low in some essential nutrients compared to legumes.The present study confirmed that fenugreek flour could be incorporated up to 10% level in the formulation of *teffinjera* without affecting their overall physical quality. In case of chemical quality *injera* containing 10 and 15% germinated and soaked fenugreek flour were the best among all the composite fenugreek flour blends *injera*. Baking quality and sensory evaluation revealed that *teff* flour can be replaced using 5 and 10% roasted and 5% germinated flours to produce acceptable *injera*. As the levels of fenugreek flour increased, the protein, mineral (calcium and zinc), fiber, fat and antioxidant activity increase whereas carbohydrate content decrease.

The proximate composition (protein, fat, and fiber) and antioxidant capacity of the product were significantly improved with increasing the proportion up to (15%) of the fenugreek flour in to *teffinjera*. Fenugreek containing *injera* sample showed significantly increased calcium and zinc content while, the iron content was found to be negatively influenced because of 100% grain *teffinjera* is known for its high iron contents. The saponin content in the *injera* was increased as the ratio of raw fenugreek flour increases up to 15% but lower in pretreated fenugreek blended samples. Thereby, *injera* samples prepared from *teff* blended with pretreated fenugreek shows lower value of phytate content and phytate: mineral molar ratio which is an indication of good bioavailability.

In the sensory evaluation *injera* prepared from *teff* and fenugreek flour blends was found to be acceptable in terms of taste, flavor, appearance and overall acceptability up to supplementation level of 10%. The 5% fenugreek and control samples rated highly acceptable in almost all attributes. Even though *injera* prepared from 15% of fenugreek flour had high nutritional content the acceptance by the society was low.

Generally, *injera* prepared from *teff* with pretreated (germinated and soaked) fenugreek blends at 5% and 10% was acceptable in chemical analysis and sensory qualities (protein 11.58 -12.27%, fiber 2.93 -3.87%, calcium 88.74 –100.23mg/100g, zinc 2.31- 3.93 mg/100g and antioxidant

activity 47.47 -57.43%. The overall acceptability of sensory attribute score in five hedonic scales was found to be in the range of 3.57 to 4.10.The phytate content was decreased about (16.60 - 19.12) which will improve the availability of minerals like iron, calcium, and zinc. While, saponin content also decrease in processed fenugreek blend *teffinjera* than raw fenugreek blends.

This study ascertained the potential of pretreated fenugreek for producing *teffinjera* to certain level. It has been shown that enrichment up to a level of 10% would produce a more nutritionally balanced and acceptable products. The results indicated that the fenugreek used in blending *teff* flour samples was able to increase the protein content, crude fiber, fat, mineral content (zinc and calcium) as well as antioxidant capacity without affecting the acceptance of the *injera*. Furthermore, the study was conducted with the broad intention of increasing the consumption of Pretreated fenugreek in the society by including it in the already established existing product this could have significant implication to Ethiopian diet. Addition of fenugreek helps to improve the protein value of *teffinjera* which are consumed by a large Ethiopian population as a staple food and also permits the exploration of the addition of fenugreek to other products to improve their nutrient composition and antioxidant capacity.

6. RECOMMENDATION

I. More research need to be done to incorporate different pretreated fenugreek flour into different cereal product to enhance nutritional values.

II. Even though high percentage (15%) of germinated fenugreek provided better nutritional composition with less anti nutritional factors as well as better antioxidant capacity, however it wasn't accepted by panellists. In order to benefit from gained nutritional and anti-nutritional increase or decrease, a flavour and taste masking strategies should be explored.

III. Further study should be done on the microbial analysis and shelf life of *injera* enriched with fenugreek seed flour to investigate either the promoting or inhibiting activity of fenugreek ingredients on mould appearance and growth.

IV. Researchers also shall focus on product development and nutrient enrichment through fortification of *teff*-fenugreek mix.

V. Further studies on fenugreek flour production technology and on the effect of cereal based products on the general well being and improvement of its nutritional status would help to promote the utilization of it.

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8. APPENDICES

Appendix Table 1 Analysis of variance p-value of proximate composition

Source	DF	MC	CP	C. fat	C. fiber	Ash	Carbohydrate	Energy
PTR	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Conc.	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PTR*Conc.	8	0.00	0.01	0.62	0.02	0.23	0.00	0.00
Error	30							

Appendix Table 2 Analysis of variance p-value of physical, chemical and functional property

Source	DF	Elasticity	Thickness	TA	PH	WAC
PTR	4	0.00	0.008	0.00	0.00	0.00
Conc.	2	0.00	0.00	0.00	0.00	0.00
PTR*Conc.	8	0.00	0.13	0.00	0.00	0.93
Error	30					

Appendix Table 3 Analysis of variance p-value mineral content

Source	DF	Iron	Zink	Calcium
PTR	4	0.0001	0.0001	0.0006
Conc.	2	0.0001	0.0001	0.0001
PTR*Conc	8	0.0001	0.0003	0.0164
Error	30			

Appendix Table 4 Analysis of variance p-value antinutritional factor and antioxidant property

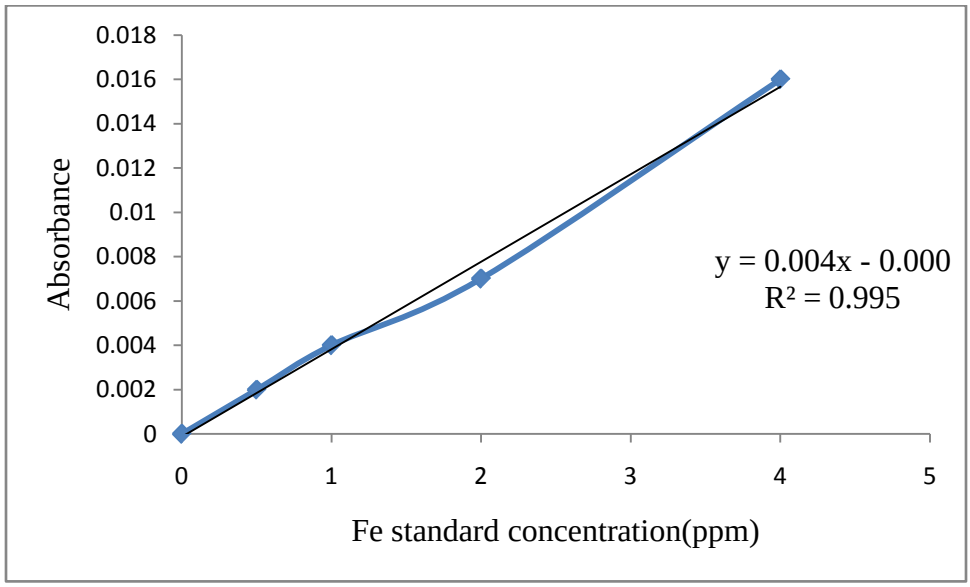
Source	DF	Saponin	Phytate	Antioxidant
PTR	4	0.000	0.000	0.000
Conc.	2	0.000	0.000	0.000
PTR*Conc	8	0.000	0.000	0.000
Error	30			

Appendix Table 5 Analysis of variance p-value of sensory evaluation

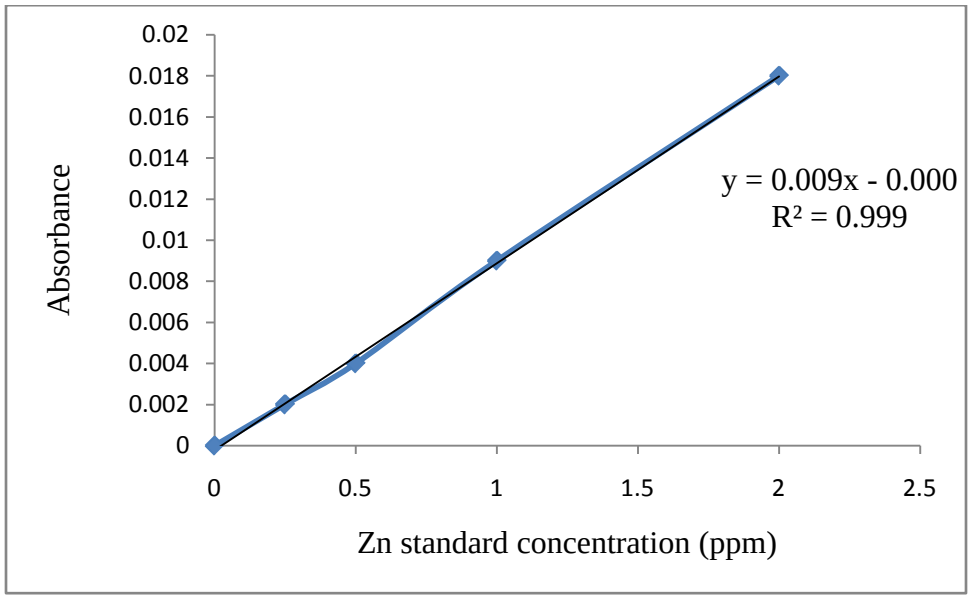
Source	DF	Colour	Flavour	Taste	Texture	Appearance	Overall acceptability
PTR	4	0.003	0.002	0.000	0.000	0.000	0.000
Conc.	2	0.000	0.000	0.000	0.001	0.000	0.000
PTR*Conc.	8	0.000	0.081	0.009	0.279	0.000	0.029
Error	30						

Appendix Figure 1 Calibration curve of Iron, Zinc and Calcium

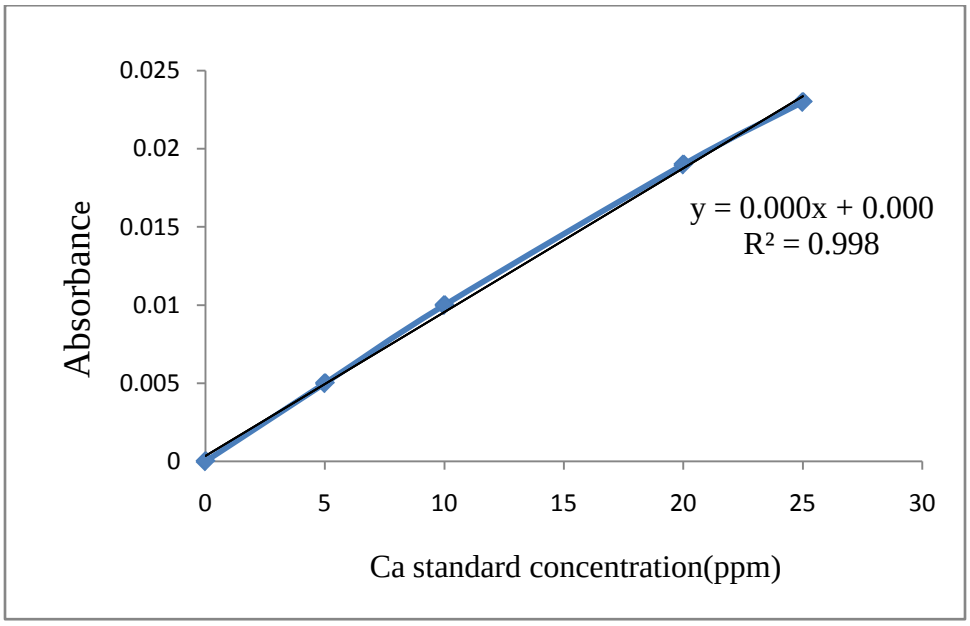
I. Iron calibration curve



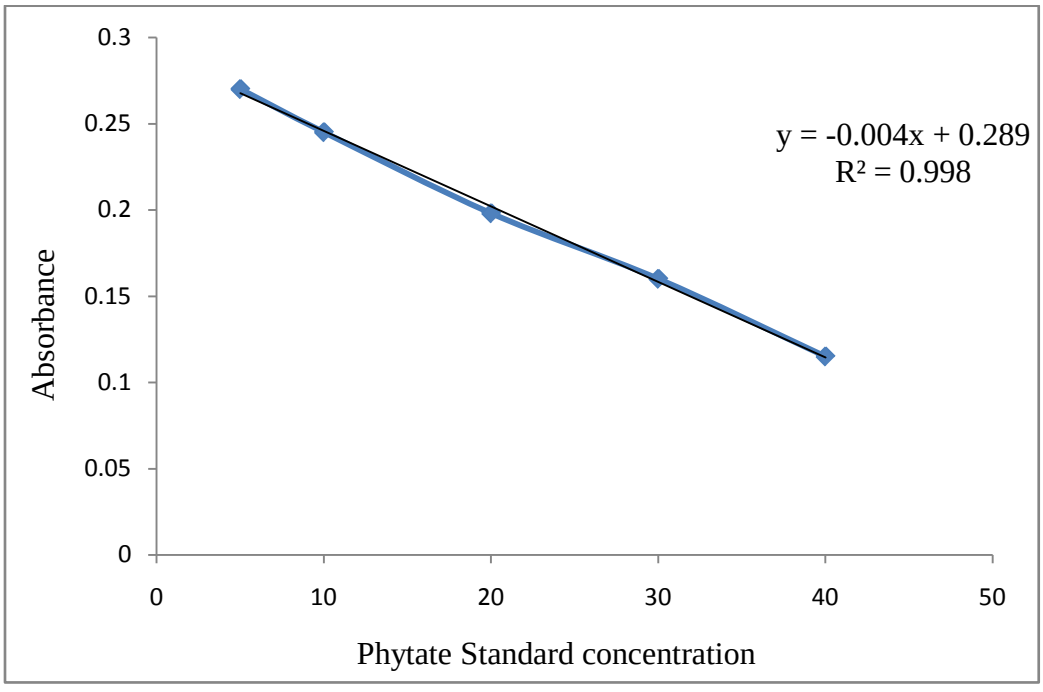
II. Zinc calibration curve



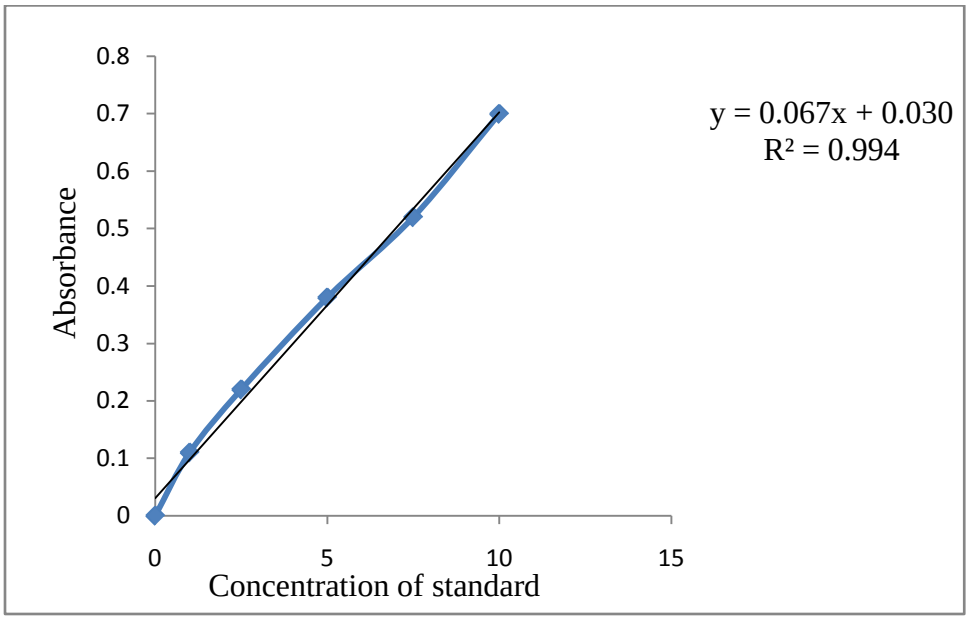
III. Calcium calibration curve



Appendix Figure 2 Calibration curve of Phytate



Appendix Figure 3 Standard curve of antioxidant



Appendix Table 6 Sensory evaluation questioner form

Please look at and taste each sample of porridge in order from left to right as shown on the ballot. Indicate how much you like or dislike each sample by checking the appropriate phrase of category which is listed below and mark your choice with the number that corresponds to your preference on each parameter.

1 (extremely dislike),

2(dislike moderately),

3 (neither like nor dislike),

4 (like moderately) and

5 (extremely like).

Sample code	Colour	Aroma	Taste	Texture	Appearance	Overall acceptability