

Organoleptic and nutritional evaluation of *Tef injera*

Supplemented with treated and untreated fenugreek

(*trigonella foenum-graecum* L.)

By:

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A Thesis submitted to the graduate program of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in food science and nutrition

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

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The image shows four horizontal lines, each with a handwritten signature in blue ink. The signatures are stylized and appear to be of the individuals listed in the text to the left.

DEDICATION

This work is dedicated to my family whom I love more than anything.

To

My father

My mother

My brothers and my sisters

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AAU	Addis Ababa University
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AR	Analytical Reagent grade
BC	Before Christ
Ca	Calcium
CaCl ₂	Calcium Chloride
CD	Celiac disease
CSA	Central Statistics Authority
DZARC	Debre Zeit Agricultural Research Center
EHNRI	Ethiopian Health and Nutrition Research Institute
ENI	Ethiopian Nutrition Institute
ENS	Ethiopian Nutritional Survey
FAO	Food and Agriculture Organization
g	gram
GFD	gluten-free diet
h	hour
ha	hectare
HCL	Hydrochloric acid
IBC	Institute of Biodiversity Conservation
Kcal	Kilocalorie
LSD	Least significant differences
Mol	Molar
M	Morality
N	Normality
NFE	Nitrogen free extract
NIN	National Institute of Nutrition
NaOH	Sodium hydro oxide

NHP	Natural Health Products
PEM	Protein energy malnutrition
phy	Phytate
SE	Standard Error
SPSS	Statistical Product and Service Solutions
UNDP	United Nations Development Programme
USA	United States of America
WFP	World food program
Zn	Zinc

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ABSTRACT

The experiment was conducted with an objective of assessing nutritional, organoleptic and antinutrient contents of fenugreek supplemented and unsupplemented Tef Injera. Therefore, Injera prepared from the blends containing different proportions (0%, 5%, 10%, 15% and 20%) of raw, roasted, soaked and germinated fenugreek seed flour were evaluated for nutritional composition and sensory characteristics. The proximate composition of fenugreek supplemented injera significantly ($p<0.05$) increased for moisture, protein, fat and fiber with blending ratio of 0%, 5% and 10%. The mean moisture, protein, fat and fiber content significantly ($p<0.05$) increased from 6.3 to 6.9 g/100g, 9.08 to 10.4 g/100g, 1.61 to 3.2 g/100g and 3.3 to 3.7 g/100g respectively. Conversely, the nutritional contents of total ash, carbohydrate, and energy generally significantly ($p<0.05$) decreased as blending proportions increased from control (0%) to 10%, from 2.5 to 2.4 g/100g for ash, 77.1 to 73.5 g/100g for carbohydrate and from 375.3 to 368.8 kca/100g for gross energy with the blending proportions of 0%, 5% and 10% respectively. Whereas sensory characteristics decreased with the increasing level of fenugreek flour. The sensory results showed that a maximum of 10% soaked and germinated fenugreek flour could be incorporated to prepare acceptable quality injera, However 5 % of all treated and untreated fenugreek flour could be added in order to make acceptable quality injera. Supplementation of raw, roasted, soaked and germinated fenugreek flour to Tef flour increased the contents of protein (10.2, 9.6, 10.5 and 11.2 g/100g), fat (3.0, 3.1, 4.4 and 4.1 g/100g), dietary fibre (3.4, 3.7, 3.3 and 2.3 g/100g), total Ca (202.17, 236.09, 447.35 and 181.05 mg/100 g) and total iron (13.39, 13.57, 11.76 and 12.44 mg/100g), respectively, at 10% level of substitution. The antinutritional factors of fenugreek supplemented injera significantly ($p<0.05$) increased for tannin 2.05 to 11.7 from control (0%) to 10%. Conversely, phytate content significantly ($p<0.05$) decreased from 150.62 to 131.28 as blending proportions increased from control (0%) to 10%. Supplementation of raw, roasted, soaked and germinated fenugreek flour to Tef flour decreased the contents of antinutritional factors tannin (11.25, 8.67, 6.64 and 4.31 mg/100g), phytate (153.46, 128.40, 144.13 and 128.32 mg/100g) respectively. Blend proportion had a significant effect ($p<0.05$) on the proximate composition of the supplemented injera. Most of the proximate composition and the sensory characteristics score were high on the soaked and germinated fenugreek supplemented injera at the same level of supplementation. In general, the study indicated that blending soaked and germinated fenugreek up to 10% and both treated and untreated fenugreek up to 5% with Tef flour could result in injera of acceptable quality.

Keywords: Antinutrients; Fenugreek; Injera; Nutritional; Sensory; Tef

INTRODUCTION

1.1 Background

Injera is thin, fermented Ethiopian traditional bread made from flour, water and starter (*Ersho*) which is a fluid saved from previously fermented dough. *Injera* is made from tef most popular grain in the country, although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used (Adamu, 1996).

Tef is believed to have originated in Ethiopia between 4000 and 1000 before Christ (BC). Nowadays, tef represents the re-discovery of a crop used by ancient civilizations (Patricia Arguedas.G, 2008). The grain has been widely cultivated and used in the countries including Ethiopia, India and Australia (Bekele, 1985).

Tef is an annual grass crop and harvested as grain not only in Ethiopia but also, at present, cultivated in South Dakota and Idaho of USA, though not widely known or used, and is available in many health food stores (Bekele, E. 1985). It is one of the main annually produced grains in Ethiopia and is about 1.32 million metric tons per year (CSA, 1998). Of note is the fact that tef commands a higher market price than other cereals in Ethiopia (Ketema, 1993).

On top of use for making *Injera*, Tef in mixtures with soybean, chickpea and other grains is used and becoming popular as baby food because of its high mineral content. The most popular and widely preferred form of injera is prepared from unmixed pure tef flour. The next preferred *Injera* is the one prepared by mixing tef flour with the flour of other cereals such as barley, wheat, maize or sorghum (Ketema, 1997).

Injera is leavened, flat round bread and its surface has essentially evenly spaced gas holes that make up a honeycomb-like structure. The holes are formed during baking due to expulsion of gas produced during fermentation. The other side (bottom surface) of *Injera* is smooth and shiny. A good *Injera* is soft, fluffy and able to be rolled without cracking. It should retain these textural

properties after two to three days of storage. Storage is traditionally done in a straw basket. A slight sourness is a characteristic taste of *Injera*. Because *Injera* is leavened bread made from no gluten containing flour and hence it has great potential for commercial production internationally (Ketema, 1997).

Injera prepared from flour of millet like grain named tef [*Eragrostis tef* (Zucc.) Trotter], is the most preferred compared to *Injera* made from other types of possible grains. Tef flour is preferred in the production of injera; a major food staple in Ethiopia (Ketema, 1997).

Injera is staple Ethiopian fermented bread which can be made from different types of cereals, particularly from tef (*Eragrostis tef*). About two-third of Ethiopian diet consists of *Injera* and it accounts about two-third of the daily protein intake of Ethiopian population. Tef is traditionally harvested for grain in Ethiopia, where its flour is used for the production of *Injera*. The nutritional value of tef grain is similar to that of traditional cereals (Norberg, 1996).

To make *Injera*, Tef is ground into flour, fermented for three days then made into injera, sourdough type flat bread. It is also eaten as porridge and used as an ingredient of home-brewed alcoholic drinks.

Some researchers suggested that fenugreek (*Trigonella foenumgraecum*) could be good supplement used with tef. In some regions of Ethiopia, e.g. Welo, women usually prepare *Injera* by adding some fenugreek to tef flour to improve its baking quality. Because of this, *Injera* is believed to become softer and possess a shiny appearance. Thus, it should be encouraged to keep on this traditional practice and users be made aware that such 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).

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). On the other hand, Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses (Basch *et al.*, 2003). Fenugreek has anti-diabetic, anti-fertility, anti-cancer, anti-microbial, antiphrastic, hypo-cholesterlaemic effects (Al-Habori and Roman, 2002). Also it contains many minerals and vitamins (Michael and Kumawat, 2003).

1.2 Statement of the problem

Malnutrition is very common problem in developing countries; the problem includes both macro and micro nutrient deficiencies. Among these Iodine disorder, Iron deficiencies and protein deficiencies are the most significant problems noted.

Ethiopia is one of the least developed countries in the world (UNDP, 2001) and is the second most severely affected by malnutrition (de Onis, 1997). The most important forms of malnutrition in Ethiopia are protein energy malnutrition (PEM), vitamin A deficiency, Iodine deficiency disorders, and Iron deficiency anemia (Edris, 2004). The daily energy intake of the average Ethiopian is lower than the WFP minimum standard (2100 calories per day). Absolute poverty, poor health and sanitary conditions, limited knowledge of nutritional matters among certain households, and fluctuations in incomes are some of the principal reasons for the high prevalence of malnutrition (Anderson, 1981). Cereals, though important in human diets as a primary source of calories, of several important vitamins and minerals, and proteins, are deficient in the essential amino acid lysine. The nutritional value of a cereal grain could therefore be improved either by addition of synthetic lysine or by dietary intake of the lysine. Food legumes are comparatively rich in lysine and therefore a combination of cereal protein and legume protein could suffice ideal source of dietary proteins for human beings. The nutritional complementarities of cereals and legumes are of extreme importance, particularly for the people of the less developed world (Hulse and Fawcett, 1975).

The processing of legume flours through different techniques such as roasting, soaking, and germination, may alter these constituents, by making it more palatable, digestible, and make them safe for human consumption by reducing the amounts of ant nutrients (Hulse and Fawcett, 1975).

Specialty legume crops like fenugreek were attracted the attention of producers to meet manufacturing demands for "functional food" additives and Natural Health Products (NHP) in Canada (Fitzpatrick, 2004). Mansour and El-Adawy (1994) recommended that fenugreek seed could be added to foods like ground meat and baked goods, not only as nutritional supplements but also as a potential functional food.

Fenugreek seed flour has a great potential to increase the protein content of tef flour products, due to its high and good quality protein, lysine, soluble and insoluble dietary fibre (Khosla et al., 1995 and Sharma, 1986). It also possesses hypocholesterolemic and hypoglycemic properties and it has high contents of Iron, calcium, zinc and other ingredients (NIN, 1987). Therefore, supplementation of fenugreek seed flour on the Tef flour enhances the nutritive value of food products. However, development and consumption of such therapeutic bakery products has not been published to help to raise the nutritional status of the Ethiopian population.

1.3 Significance of the study

Supplementation is important to improve the nutritional quality of food. It plays a noteworthy role in the new product production and health area. Consumers and researchers are required to give a detailed attention to this in order to minimize malnutrition. It is believed that various bodies will benefit from the results of this study. Researchers in the supplementation area, teachers, students, and food processing industries may use the outcome of the research as a reference material or a base line data for further research in the same area. It also provide necessary information about the nutritional and acceptance of the developed product. And help to change the quality and nutritional status of Ethiopian *Tef Injera* and to save considerable amount of expenses in health and medical services due to consumption of inadequately prepared food.

Therefore, it was desirable to design and study the effect of supplementation of *Tef* flour by treated and untreated fenugreek seed flours on nutritional and sensory characteristics and of produced injera so as to curtail problems associated with malnutrition in the country. Hence, this study was conducted with the following general and specific objectives.

1.4 Objectives

1.4.1. General objectives

- To study the effect of supplementation of treated and untreated fenugreek to tef flours on organoleptic and nutritional value of *Injera*.

1.4.2. Specific objective

1. To determine nutritional contents of fenugreek supplemented and unsupplemented *Tef Injera*.
2. To characterize organoleptic properties of the fenugreek supplemented *Tef Injera*.
3. To identify antinutrient contents of fenugreek supplemented *Tef Injera*.

Literature Review

2.1 Production of cereals and legumes in Ethiopia

Ethiopia is one of the richest genetic resource centers in the world in terms of crop diversity. This is principally attributed to the diverse farming systems, socio-economics, cultures and agroecologies. Crop plants such as coffee (*Coffea arabica*), safflower (*Carthamus tinctorius*), tef (*Eragrostis tef*), noug (*Guizotia abyssinica*), anchote (*Coccinia abyssinica*), enset (*Ensete ventricosum*), are known to have originated in Ethiopia. Very high genetic diversity is found in Ethiopia in four of the world's widely grown food crops (wheat, barley, sorghum and peas); in three of the world's most important industrial crops (linseed, castor and cotton); in the world's most important cash crop (coffee); in a number of food crops of regional and local importance (tef, noug, Ethiopian mustard, enset, finger millet, cowpea, lentil) and in a number of forage species of world importance (clovers, medics, oats)(IBC, 2008).

The Ethiopian region is characterized by a wide range of agro-climatic conditions with diverse cultural and farming practices that can be grouped into three major agricultural systems: the highland mixed farming system, the low plateaux and valley mixed agriculture and the pastoral livestock production of the arid and semi-arid zones. Agriculture in Ethiopia is a basis for the entire socioeconomic structure of the country and has a major influence on all other economic sectors and development processes of the country. It provides about 85 percent of the total employment and generates about 40 percent of the country's earnings from export, contributing about 57 percent of the country GDP and 90 percent of the export earnings. Of the total area, which is 122 million hectares, 84 million hectares (69 percent) is classified as agricultural land suitable for crop and livestock production. Of this, about 32 million hectares is cultivated with about 12 million hectares under major crop production annually (IBC, 2008).

At present approximately 8 million ha is used to produce the main food crops: cereals, legumes and oilseeds .Cereals [tef (*Eragrostis tef*), maize, barley, wheat and sorghum] occupy 80 to 85% of the total cultivated area, followed by legumes and oil seeds. The area annually covered by major cereal crops are 2.1, 1.6, 1.4, 0.9 and 0.8 million ha for tef, maize, sorghum, wheat and barley, respectively. Pulses (faba bean, field peas, haricot bean, chickpea, grass pea, lentil) occupy 1 million ha and oilseed crops [niger seed (*Guzotia abyssinica*), linseed, rapeseed, peanut, sunflower, sesame and castor bean] about 0.4 million ha (Getinet, 2001).

Table 2.1 Area and production of major crops from 1997/98 to 1998/99

Crops	Area in ha		Production in tonnes		Productivity in tonnes	
	1997/98	1998/99	1997/98	1998/99	1997/98	1998/99
Cereals	5601.88	6744.73	6498.80	7683.00	1.16	1.14
Legumes	837.60	875.38	680.20	931.98	0.81	0.84
Oil seeds	383.50	374.78	164.49	156.75	0.45	0.43
Others	29.71	21.43	16.22	12.13	0.55	0.57
All crops	6852.69	8016.32	9645.23	7359.71	1.07	1.07

Source: Central Statistics Authority (CSA), Bulletin Nos. 171 (1997), 189 (1998) and 200 (1999)

Cereal crops play a major role in Ethiopian agriculture. The recent data from CSA indicates that from the total cultivated of 9.8 million hectare, 7.6 million hectare is covered by cereals, accounting for 77 percent of the total cultivated area and 84 percent of the total agricultural production. The major indigenous cereal crops grown in Ethiopia are tef, durum wheat, barley and sorghum. Tef is the most important staple food crop in Ethiopia (IBC, 2008).

The versatility of tef, the relatively high market price for its grain and straw make it one of the very few cereal crops which play a very important role in the livelihoods of Ethiopian farmers. Apart from its importance locally, official figures in 1997/98 and 1998/99 indicated that 9 million and 14.6 million birr, respectively, were obtained from the export of tef. The trend indicates that there is a good export market for this crop in the Middle East, North America and Europe, mainly for the immigrant Ethiopians. The IBC recently signed an agreement with a Netherlands food company interested in commercialization of tef products. As tef is an indigenous cereal to Ethiopia, most of the available genetic variation has been generated naturally and through crop improvement research. Germplasm collections that exist cover new and old varieties, landraces, breeding lines and related wild species (IBC, 2008).

Ethiopia is one of the major centers of diversity of grain legume crops, like field pea, grass pea, chickpea, lentil and wild species of cowpea. In Ethiopia, pulses are next to cereals in terms of production and are cheap sources of protein. They also play an increasingly important role in the export market. The total allotted area for the production of pulses is nearly 1.4 million ha, accounting for 13 percent of the total cultivated area and 11 percent of the total grain production (IBC, 2008).

2.2 Cereals in human diets

Cereals are important in human diets as a primary source of calories, of several important vitamins and minerals, and of protein. "Protein" is the name given to a class of organic substances which are made up of linked amino acids composed of nitrogen and other elements. Amino acids are the essential building materials of which all animals' tissues and organs are composed and maintained. Protein is essential to the growth and restoration of body tissue and is particularly important in the diets of young children, pregnant and lactating women, and to aid recovery after serious illness or injury. There is evidence to suggest that serious protein

deficiency in early childhood can impede brain development and learning ability. Different proteins are composed of different combinations of amino acids. Some amino acids can be synthesized by living organisms from other sources of organic and inorganic nitrogen. The amino acids which can be synthesized vary among different higher animals. Amino acids essential to the animals' diet which cannot be synthesized but must be ingested as such are known as "essential" amino acids and must be provided in the food which is eaten.

Human beings require at least 10 essential amino acids and when these 10 amino acids appear together in appropriate proportions in a single protein source, that source is described as an "ideal" or "perfect" source of protein. Though the protein content of cereals varies widely, for example on a dry weight basis, the protein content of wheat from different sources has been reported to vary from 7% to 26%; almost all cereal proteins are deficient in the essential amino acid lysine. The nutritional value of a cereal grain can be therefore improved by the addition of synthetic lysine or by the addition of a food which is rich in lysine. One can list a great many protein foods of animal, vegetable and microbiological origin which are rich in lysine and which might be used to supplement cereals (Hulse and Fawcett, 1975).

2.3 Food Legumes

The botanical family Luminosac, characterized by the fact that their seeds are contained in pods, is one of the largest families of flowering plants comprising nearly 700 genera and 18,000 species. Legumes which are eaten by humans are usually called food legumes or grain legumes and these may be partitioned into two sub-groups, the pulses and the oilseeds. The pulses are the dried edible seeds of cultivated legumes, whereas the oilseeds, as their name suggests, are sources of both edible oil and protein.

The food legumes are eaten in a wide variety of ways. The green seeds may be cooked as vegetables; they may be dried, parched or toasted; they may be ground into flour and cooked as

such or mixed with cereal flours; they may be fermented or allowed to germinate; they may be modified chemically or microbiologically to produce bean curds and pastes. From soybean, groundnut and sesame, the oil may be extracted by pressing or by solvent extraction. In many countries the high protein residue following oil extraction is usually fed to animals, but recently processing methods have been developed whereby to extract high protein isolates from the oil-free meal for subsequent conversion to a variety of edible forms. It is difficult to compare the cost of different sources of protein largely because of the highly variable cost of production among different countries and different economic systems. However, as a rough rule of thumb, one can say that the cost per kilogram of utilizable protein from meat or meat by-products is about 10 times the cost of a kilo of utilizable protein from an edible legume.

Food legumes are comparatively rich in lysine than cereals and therefore a combination of cereal protein and legume protein comes very close to providing an ideal source of dietary proteins for human beings. The nutritional complementarities of cereals and legumes are of extreme importance, particularly for the people of the less developed world (Hulse and Fawcett, 1975).

2.4 Antinutritional factors

Cereals and legumes are often rich in fibre-associated anti-nutritional factors (namely phytate, polyphenols, oxalate) (Frolich, 1995) that reduce the bioavailability of minerals. The bioavailability of a nutrient is defined as the proportion of the total nutrient content in a food, meal or diet that is utilized for normal metabolic functions. Many minerals and trace elements are inefficiently and variably absorbed from the diet, for instance iron (<1–30%) and zinc (<15–50%). This phenomenon must be taken into consideration in the preparation of complementary foods in developing countries, where young children often suffer from micro-nutrient deficiencies, such as anemia, caused by iron deficiency (Hurrell, 1997) or decreased growth rate due to zinc deficiency (Gibson & Ferguson, 1998).

2.4.1 Classification of the anti-nutritional factors

The anti-nutritional factors in plants may be classified on the basis of their chemical structure, the specific actions they bring about or their biosynthetic origin (Aletor, 1999). Although this classification does not encompass all the known groups of anti-nutritional factors, it does present the list of those frequently found in human foods and animal feedstuffs. The anti-nutritional factors may be divided into two major categories. They are:

- (1). Proteins (such as lectins and protease inhibitors) which are sensitive to normal processing temperatures.
- (2). Other substances which are stable or resistant to these temperatures and which include, among many others, polyphenolic compounds (mainly condensed tannins), non-protein amino acids and galactomannan gums (Osagie, 1998).

More often than not, a single plant may contain two or more toxic compounds, generally drawn from the two categories, which add to the difficulties of detoxification. According to Aletor (1993), there are several antinutritional factors that are very significant in plants used for human foods and animal feeds. They are:

- (i) Enzyme inhibitors (trypsin and chymotrypsin inhibitors, plasmin inhibitors, elastase inhibitors)
- (ii) Haemagglutinins (concanavalin A, ricin)
- (iii) Plant enzymes (urease, lipoxygenase),
- (iv) Cyanogenic glycosides (phaseolunatin, dhurrin, linamarin, luteosyringin),
- (v) Goitrogens (pro-goitrins and glucosinolates),
- (vi) Oestrogens (flavones and genistein),
- (vii) Saponins (soyapogenin),
- (viii) Gossypol from *Gossypium species* e.g. cotton,

- (ix) Tannins (condensed and hydrolysable tannins),
- (x) Amino acid analogues (BOAA, DAP, mimosine, N-methyl-1-alanine),
- (xi) Alkaloids (solanine and chaconine),
- (xii) Anti-metals (phytates and oxalates),
- (xiii) Anti-vitamins (anti-vitamins A, D, E and B12) and
- (xiv) Favism factors.

2.4.1.1 Tannins

Tannins are secondary compounds of various chemical structures widely occurring in plant kingdom (Francis *et al.*, 2001). They are defined as high-molecular-weight polyphenolic compounds that have the ability to bind with protein and preserve animal hides. However, the term “tannin” is commonly used to refer polyphenolic compounds. Tannins are generally divided into hydrolysable (glucose polyesters of Gallic or hexahydroxydiphenic acids) and condensed tannins (proanthocyanidins) (Bender, 2006; Maxson and Rooney, 1972). Their anti-nutritional effects include interference with the digestive processes either by binding the enzymes or by binding to food components like proteins or minerals. Tannins also reduce the absorption of vitamin B12. Contrary to condensed tannins, the hydrolysable tannins are easily degraded in biological systems, forming smaller compounds that can enter the blood stream and over a period of time cause toxicity to the organs (e.g., liver and kidney). Tannins are also known to interact with other anti-nutrients. For example, interaction between tannins and lectins removed the inhibitory action of tannins on amylase, and interactions between tannins and cyanogenic glycosides reduced the deleterious effects of the latter (Francis *et al.*, 2001). Condensed tannin levels in grass pea lines ranged from zero to 4.38 g/kg. Tannins are strongly astringent (owing to their protein-binding properties), a depression of feed intake, which lowers animal productivity, would be expected. Although astringency seems to be the major cause of lower feed intakes,

several other factors may contribute to the lower feed efficiency ratios of tannin-containing diets. These include the formation of tannin/protein complexes that make the protein unavailable, inhibition of the digestive enzymes, increased synthesis of digestive enzymes due to inadequate enzyme digestion, and increased loss of endogenous proteins such as the mucoproteins of the gastrointestinal tract (Cambell, 1997).

2.4.1.2 Phytic acid

Phytate is especially known as a chelating agent that reduces the bioavailability of divalent cations (Weaver & Kannan, 2002). Phytic acid (*myo*-inositol hexaphosphate) is a constituent of plants such as seeds. Phytates are the primary storage form of both phosphate and inositol in plant seeds and grains. Phytic acid is formed during maturation of the plant seed. In dormant seeds, it represents 60–90 % of the total phosphate. Phytate is, therefore, a common constituent of plant-derived foods (Melaku, 2005). Depending on the amount of plant-derived foods in the diet and the level of food processing, the daily intake of phytate is estimated between 2000–2600mg for vegetarian diets and diets of inhabitants of rural areas in developing countries, and about 150–1400 mg for mixed diets (Greiner, 2006). Fiber rich foods, including cereals like sorghum, contain high levels of phytic acid. It is usually located in the outer layer of cereals where the fiber is primarily concentrated. In many developing countries, diets based on legumes and cereals will contain up to 600mg phytate per day compared to 170 mg per day in North American diets. Phytic acid is highest in whole-grain flours and can be decreased considerably by removing its hull (Hurrell, 2003).

Phytic acid form complex compounds with cations such as zinc, iron, magnesium and calcium at physiological pH. Phytates are complex compound between phytic acid and cations. It is either insoluble or difficult to hydrolyze during digestion. Phytic acid inhibits minerals (such as iron, zinc) absorption by humans and other monogastric animals in a dose-dependent manner. When

the molar ratio of phytate: iron and phytate: zinc is above 0.15 and 15, respectively, phytate inhibits absorption of minerals (Melaku ,2005).

The antinutritional effect of phytic acid is primarily related to its strong chelating ability with minerals and proteins. It is intern associated with its six reactive phosphate groups. Multivalent cations are particularly susceptible and form insoluble, indigestible complexes. Phytates also affect enzyme activity with a negative effect for key digestive enzymes including amylase, pepsin and trypsin (Greiner, 2006). The interaction between phytate and proteins leads to decreased solubility of proteins. This is due to the fact that phytic acid-protein complexes are less subjected to proteolytic attack than the same protein alone. The reduced solubility of proteins may adversely affect certain functional properties of protein. Phytic acid can be degraded in cereal-based weaning foods by adding commercial exogenous phytases or by activating the native phytases by a combination of soaking, germination, and fermentation (Hurrell, 2003).

2.5 Processing methods

Certain biological or thermal treatments, such as appertisation, allow phytate content to be reduced. The most effective treatments are fermentation and germination but their application remains limited because of the additional workload they imply or the particular organoleptic properties they induce. Soaking is a simple technological treatment that is often used by mothers to prepare complementary foods at home. Moreover, it can be a simple prolongation of the obligatory washing of the seeds and can also have other advantages, such as facilitating dehulling or swelling of seeds. Previous studies have shown that a long soaking period before fermentation or germination, leads to a reduction in phytate content and to an enhancement of mineral HCl-extractability, used to estimate mineral bioavailability (Duhan *et al.*, 2002; Sandberg & Svanberg, 1991).

2.6 Production of tef in Ethiopia

Tef is endemic to Ethiopia and its major diversity is found only in that country as with several other crops, the exact date and location for the domestication of tef is unknown. However, there is no doubt that it is a very ancient crop in Ethiopia, where domestication took place before the birth of Christ. According to Ponti (1978) tef was introduced to Ethiopia well before the Semitic invasion of 1000 to 4000 BC. It was probably cultivated in Ethiopia even before the ancient introduction of emmer and barley. According to Tadesse (1975) tef seeds found by Unger (1866) in the Pyramid of Dashur (3359 BC) and from the ancient Jewish town of Ramses in Egypt (ca. 1300 BC) were probably *E. aegyptiaca* or *E. pilosa* and thus are not good evidence for the cultivation of tef in ancient Egypt.



Figure 2.1 Tef on farm

Tef is adapted to a wide range of climatic and agricultural conditions. It can be grown from sea level up to 2800 m of altitude, under different temperatures, soil conditions, and rainfall. Tef grains are very small: 1000 grains weigh 0.3 – 0.4 g. Their color varies from white to dark brown, with the lighter the color being the more expensive (Ketema, 1997). For example, white tef grows only in the Highlands and will thrive only under these ideal environmental conditions, and then only through a great deal of effort. Particularly in poor households tef is often mixed with wheat because of its lower price (Piccinin, 2002).

Tef (*Eragrostis tef* (Zucc.) Trotter), a traditional crop that grows very well under various stress conditions and is extensively used in Ethiopia, but little known elsewhere, is the subject of this monograph. Tef is an important cereal crop in Ethiopia. The area under tef cultivation is over one million hectares of land each year. During the 1994/95 cropping season tef occupied 32% of the cultivated land under cereals, while maize occupied 19%, sorghum 16%, barley 15%, wheat 13%, millet 4% and oats 1% (CSA 1995); this is similar to previous production years and clearly shows the importance of tef in Ethiopia. Outside Ethiopia there is a growing interest in using tef. For example, small scale commercial production of tef has begun in a few areas of the wheat belts of the USA, Canada and Australia. Tef has been introduced to South Africa and cultivated as a forage crop, and in recent years cultivated as a cereal crop in northern Kenya.

2.6.1 Taxonomy and Vernacular Name of Tef

Taxonomically, tef [*Eragrostis tef* (Zucc.) Trotter], belongs to the Kingdom –*plantae*, order – *Poales*, grass family- *poaceae*, subfamily – *eragrostidae*, tribe- *eragrostae*, genus - *eragrostis* and species - *E.tef*. The genus *Eragrostis* includes about 300 species but only a few are of significant agricultural value (Ketema ,1993).The common vernacular name of the crop in Ethiopia is ‘tef’ in Amharic, ‘*taafi*’ in Afaan Oromoo and ‘*taf*’ in Tigrigna.

2.6.2 Types of Tef

There are several varieties of Tef, each with characteristics best suited to specific conditions. It is not in the scope of this paper to discuss the details about all the different varieties of this grain. Although there is no unifying classification of the tef type throughout the country, in general, there are three main types of tef: white, red and mixed (sergegna). Tef is the smallest grain in the world. White tef is the preferred type but only grows in certain regions of Ethiopia. White tef grows only in the Highlands of Ethiopia, requires the most rigorous growing conditions, and is the most expensive form of tef. The prestige associated with consuming white tef, as well as its more stringent growing conditions, contributes to the increased cost of white tef. The shelf life of Enjera is extended with the use of white tef (Mesfin, 2004).

Red tef is the least expensive form and the least preferred type, has the highest iron content. In persons living in areas of the country where consumption of red tef is most prevalent, hemoglobin levels were found to be higher with a decreased risk of anemia related to parasitic infection (Mengesha, 1966). According to the studies, due to the increased health benefits associated with high iron contents in red tef, there is more acceptance of this grain in the society. Today in Ethiopia, red tef is becoming more popular related to its increased iron content. However, few researchers indicate that the level of iron in the tef is related to the threshing of the grain on the soil.

The third main type of tef, brown (mixed) tef is a mixture of both red and white tef without any standard proportions from each and locally it is called “mixed” tef (Mesfin, 2004). Wholesalers in the major markets divide tef into four, namely; magna (very white), nech (white), mixed (mixed between white and red) and key. Four of the grades indicate the color of the tef variety. The market value gives magna the first while key the last grade position (Refera, 2009). Poor

farmers usually sell the white tef at reasonably higher prices, while buying either the mixed or red tef for their own consumption. White tef is more expensive than the red tef. People who can afford the price usually consume white tef or Magna tef, while the medium and poor families consume the mixed and/or red tef. Farmers who grow white tef often do not consume it. Instead, they sell the white tef at reasonably higher prices and buy either the red or the mixed tef for home consumption. According to the pilot study made by (Kinde Kassegne, 2009), the classification of tef is not uniform, what is referred to as *magna tef* (top quality) in some area of Ethiopia is sometimes sold as *mixed tef* (medium quality) in Addis Ababa commodity market. Quality attributes required in tef appear to be poorly understood with little documentation of quality requirements for products. This introduces some arbitrariness in the comparison of prices at different locations (Zelege, 2009).

2.6.3 Uses of Tef as Food

Tef is produced only in Ethiopia as a grain crop; hence, no studies will be referred from other countries experience. In Ethiopia, it is traditionally grown as a cereal crop. It provides over two-thirds of the human nutrition in Ethiopia (Belay, 2005). The grain is ground into flour that is mainly used for making popular pancake-like local bread possibly with many eyes called *Enjera* 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. *Enjera* is then, fried into large flat pancakes, done on a specialized electric stove or ‘*Mitad*’ or ‘*Eele*’ on fire. *Enjera* made from tef is traditionally consumed with a variety of stews such as *wot*, a sauce made of meat or ground pulses like lentil, faba bean, field pea, broad bean and chickpea. Since *wot*, supplements the lysine deficit in tef and provides a better balanced diet, the traditional way of consuming tef

with *wot* is wise. Because of its high mineral content, the need for tef grain is increasing to be used in mixtures with soybean, chickpea and other grains in the baby food industry (FAO, 2008). The grain is also used to make local alcoholic drinks, called *tela or farsso* and *katikala*. Nowadays, efforts are being made to discover and introduce other methods of utilizing tef. Cooked tef is a good thickener for soups, stews, and gravies. Its mild, slightly molasses-like sweetness makes tef easy to include. It is also grown on a limited basis for livestock forage in other parts of Africa, India, Australia and USA. In the U.S. small acreages of tef are grown for grain production and sold to Ethiopian restaurants or utilized as a late planted livestock forage (Belay, 2005).



Figure 2.2 Tef injera

2.6.4 Nutritional status of Tef

Tef is well known by Ethiopians for its superior nutritional quality. It provides about two-thirds of the daily dietary protein intake of most Ethiopians (Besrat,1980) .Nutritionally, whole grain tef per 100 g edible portion contains water 11.0 g, Energy 357.6 kcal, Protein 9.6 g, Carbohydrate 74.6 g , fat 2.53 g ,fiber 2.63.0 g, Thiamine 0.3 mg, riboflavin 0.2 mg Niacin 2.5 mg and ascorbic acid 88 mg (EHNRI ,1997). It is an excellent source of essential amino acids-

lysine, and the amino acid that is most often deficient in grain foods. *Tef* contains more lysine than barley, millet, and wheat and slightly less than rice or oats and it is gluten free. Besides providing protein and Calories, tef is a good source of minerals particularly Iron. Compared with other cereals, *Tef* is reported to have higher iron content (EHNRI, 1997).

Tef is also an excellent source of fiber, and has many times amount of calcium, potassium and other essential minerals found in an equal amount of other grains. The iron content of tef has been controversial. The relatively high values of iron reported in, according to some studies (Besrat, 1980), and is attributed to extraneous contaminants it receives from soil during threshing and not due to high intrinsic grain contents. Whether derived from contamination or from inherent grain contents, however, the higher iron intake from consumption of tef than non-tef consumers is unequivocal (ENS, 1959). This can be confirmed from the fact that non-tef consumers have a lower level of hemoglobin, and hookworm anemia develops in non-tef eaters if they are infested with hookworm. On the other hand, since tef eaters have higher levels of hemoglobin in their blood, they do not suffer from hookworm anemia even when infested. Malaria is also frequently 'found in the groups with lower hemoglobin levels. Moreover, consuming tef is reported to prevent the anemia related to pregnancy (Ebba, 1969).

When tef is used to make *Enjera*, a short fermentation process allows the yeast to generate more vitamins (ENS, 1959). Fermentation of tef flour decreases the pH of the dough with partial breakdown of soluble starch. This also increases the dialyzable portions of its Fe, P and Zn contents. The increase in dialyzable Fe results in chance for the bioavailability (Kinde Kassegne, 2009). The fermentation process increases the bioavailability of metals. Hence, reduces iron deficiency anemia, among tef-*Enjera* consuming peoples. However, a longer duration of fermentation decreases the protein quality of *Enjera*. In some regions of Ethiopia, women usually prepare *Enjera* by adding some fenugreek to tef to improve its baking quality. Because of

this, the *Enjera* 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 *Enjera* but also of supplementing its protein content, especially lysine (Ketema, 1997).

Tef also have applications for persons with Celiac Disease. It is nearly gluten-free, and because of this gaining popularity in the whole food and Health food industry in Netherlands as an alternative grain for persons with gluten sensitivity. Celiac disease (CD) is a multifactorial disease with a strong genetic association. It is caused by a T-cell mediated immune response to wheat gluten. The treatment is a strict

gluten-free diet (GFD). Symptoms include chronic diarrhoea, failure to thrive (in children), and fatigue. Celiac (Greek, *coeliac* -abdominal) disease is caused by a reaction to gliadin a gluten protein found in wheat (and similar proteins of such as barley and rye). Upon exposure to gliadin, the enzyme tissue trans- glutaminase modifies the protein, and the immune system cross-reacts with the bowel tissue, causing an inflammatory reaction. That leads to flattening of the lining of the small intestine. This interferes with the absorption of nutrients because the intestinal villi are responsible for absorption. The only effective treatment is a lifelong gluten-free diet and tef is one of or may be the only such diet (Hopman , 2008).

2.7 Status of fenugreek

Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage legume crop. The species name "*foenum-graecum* "means "Greek hay" indicating its use as a forage crop in the past. Fenugreek is believed to be native to the Mediterranean region (Petropoulos, 2002), but now is grown as a spice in most parts of the world. It is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, Argentina, Canada, United States of America (USA) and Australia

(Fazli and Hardman, 1968; Petropoulos, 2002). India is the leading fenugreek producing country in the world (Edison, 1995).

Fenugreek is regarded as the oldest known medicinal plant in recorded history (Lust, 1986). Its seed and leaves have medicinal value, and have been used to reduce blood sugar and lower blood cholesterol in humans and animals (Dahanukar *et al.*, 2000). In parts of Asia, the young plants are used as "pot herbs" and the seed as a spice or herbal medicine. Fenugreek has many features that will benefit Canada's agri-food industry, one of them being its potential for use as a forage crop. The crop is adapted to rain-fed growing conditions and should grow well within the semi-arid regions of western Canada. It is a legume and can be incorporated into short term crop rotations (Moyer *et al.*, 2003) to replenish nitrogen within the soil. It also can produce high quantity (Mir *et al.*, 1993) and high quality forage, can be grown for hay or silage (Mir *et al.*, 1998), does not cause bloat in cattle, and contains animal growth promoting substances such as diosgenin not present in other forage legumes (Mir *et al.*, 1997).

2.7.1 Origin, distribution and different species

Different authors have widely divergent opinions about the probable ancestry of *T. foenum-graecum*. Vavilov (1926) has suggested that fenugreek is native to the Mediterranean region, while De Candolle (1964) and Fazli and Hardman (1968) proposed an Asian origin for the crop and Dangi *et al.* (2004) suggested that *T. caerulea* and *T. foenum-graecum* both originated in Turkey. Confusion over the origins of fenugreek led Sinskaya (1961) to suggest that locating the direct wild ancestor of *T. foenum-graecum* is debatable. Indigenous species have been reported (Allen and Allen, 1981; Ivimey-Cook, 1968; Petropoulos, 2002; Phitos and Damboldt, 1985; Polunin, 1988) on the continents of Asia (6 species), Europe (5 species), Africa (1 species) and Australia (1 species). Fenugreek also is reported as a cultivated crop in parts of Europe, northern

Africa, west and south Asia, North and South America and Australia (Edison, 1995; Fazli and Hardman, 1968; Jongebloed, 2004; Petropoulos, 2002; Rosengarten, 1969; Rouk and Mangesha, 1963; Smith, 1982).

Table 2.2 Distribution of fenugreek (*Trigonella foenum-graecum* L.) by country and continent.

Continents	Country(s)
Europe	Austria, France, Germany, Greece, Portugal, Russia, Spain, Switzerland, Turkey and United Kingdom (UK)
Africa	Egypt, Ethiopia, Kenya, Morocco, Sudan, Tanzania and Tunisia
Asia	China, India, Iran, Israel, Japan, Lebanon and Pakistan
South America	Argentina
North America	Canada and the United States
Australia	Parts of Australia

Source: - seed production technology for fenugreek (Saikat, 2006)

The exact number of species of fenugreek also has been debated. Petropoulos (2002) indicated that older taxonomists like Linnaeus have suggested that as many as 260 species of fenugreek exist. In contrast, about 128 species of fenugreek were reported by Vasil'chenko (1953), 97 by Fazli (1967) and 70 by Hector (1936), Hutchinson (1964) and, Rouk and Mangesha (1963). A total of 18 different species of fenugreek (*Trigonella*) currently are recognized in the primary literature.



din3945 www.fotosearch.com
 Figure 2.3 Fresh fenugreek



1641351 www.fotosearch.com
 Figure 2.4 Bowl of Dried fenugreek

Table 2.3 Common species of the *Trigonella* genus according to Petropoulos (2002).

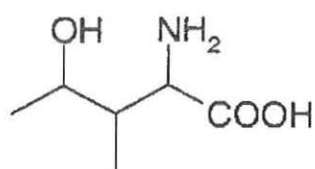
Genus	Species
<i>Trigonella</i>	<i>T. foenum-graecum</i> , <i>T. anguina</i> , <i>T. arabica</i> , <i>T. caerulea</i> , <i>T. corniculata</i> , <i>T. cariensis</i> , <i>T. rigida</i> ,, <i>T. suavissima</i> , <i>T. torulosa</i> , <i>T. spinosa</i> , <i>T. polycerata</i> , <i>T. radiata</i> , <i>T. platycarpus</i> , <i>T. hamosa</i> , <i>T. cretica</i> , <i>T. occulta</i> , <i>T. arcuata</i> , and <i>T. striata</i>

Source: - seed production technology for fenugreek (Saikat, 2006)

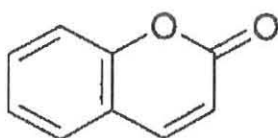
2.7.2 Functional food aspects

Composition of Fenugreek- Fenugreek has been studied very carefully for its composition. It contains about 30% protein, which has a Net Protein Utilization value of 64, coming close to meat. It contains about 45% carbohydrates all of which are in the form of soluble and insoluble fiber. It also contains minerals which are beneficial to humans. Fenugreek seed is very bitter, and also has a strong flavor. These are due to the two alkaloids as well as volatile fatty acid. The plant uses these bitterness and strong flavor to repel herbivorous animals that may harm the propagation of the species. The extracts of Fenugreek are a mixture of plant saponins and steroids, alkaloids such as choline and trigonellin, and the essential fat (Robert, 1994).

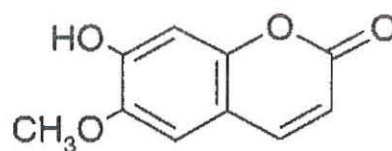
Legume consumption is known to have a beneficial or protective effect in diabetes, hypercholesterolaemia and coronary heart disease, as well as protecting against obesity and menopause (Mazur *et al.*, 1998; Madar and Stark, 2002). Specialty crops like fenugreek are attracting the attention of producers to meet manufacturing demands for "functional food" additives and Natural Health Products (NHP) in Canada (Fitzpatrick, 2004). Mansour and El-Adawy (1994) recommended that fenugreek seed could be added to foods like ground meat and baked goods, not only as nutritional supplements but also as a potential functional food. A summary showing studies associated with functional foods developed with fenugreek additives is presented in Table 2.4.



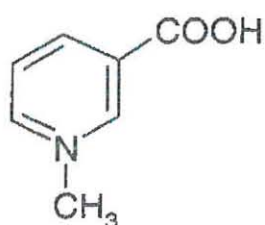
A.



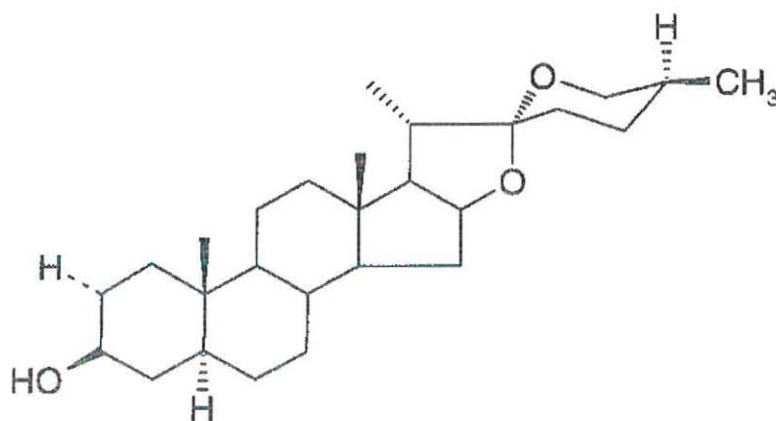
B.



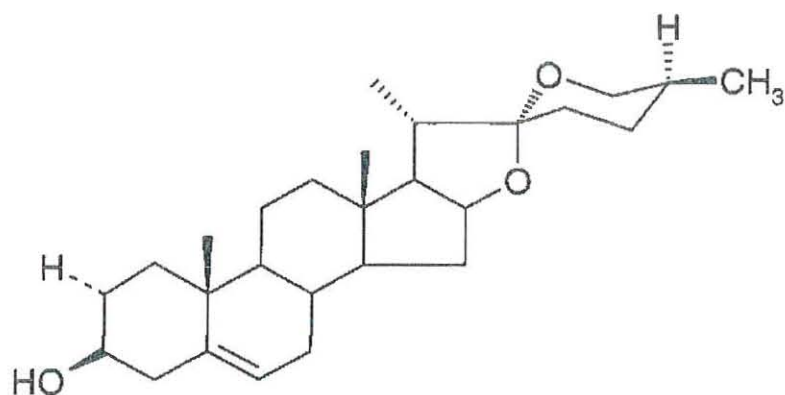
C.



D.



E.



F.

Figure 2.5 Structures of important chemical constituents detected in fenugreek

A. 4- Hydroxyisoleucine; B.Coumarin; C. Scopoletin; D. Trigonelline; E. Tigogenin and F. Diosgenin.

Table 2.4 Summary of research progress with respect to the functional food aspect of fenugreek.

Functional food aspect of fenugreek	References
Fenugreek seed has protein, lipid, cellulose starch and ash. It is also reported to be rich in calcium, iron and P-carotene.	Sauvaire <i>et al.</i> (1976)
Fenugreek seed endosperm is characterized by soluble fiber. Approximately half the dry weight of fenugreek seed has Soluble Dietary Fibers (SDF), an edible dietary fiber	Aspinall (1980)
Boiling of fenugreek seed results in loss of crude proteins, total sugars, ash but subsequently increases the level of crude fibers; without any significant effect on lipid levels.	Ismail (1996)
Good quality wheat breads with high nutritional characteristics and higher acceptability have been produced in Egypt by supplementation of 4 % fenugreek flour.	Bakr (1997)
Cooking fenugreek leaves in a pressure cooker improve retention of essential chemical nutrients such as ascorbic acid and beta-carotene	Yadav and Sehgal (1997)
Fenugreek galactomanans as food emulsifiers	Gatri <i>et al.</i> (1997)
Fenugreek seed contain sufficient ascorbic acid	Riddochefa/. (1998)
Nutritional quality of lactic acid fermented fenugreek leaves	Gupta <i>et al.</i> (1998)
Fenugreek fibers, psyllium husk and wheat bran could be used as dietary supplements to increase roughage in the human diet	Al-Khalidi <i>et al.</i> (1999)
Supplementation of wheat flour with ground debittered fenugreek improves the physicochemical, nutritional and rheological properties	Sharma and Chauhan (2000)
Suitability of the development of food products based on millets, legumes and fenugreek seed in diabetic diet	Pathak <i>et al</i> (2000)
Use of corn bread mixed with a small amount of fenugreek (3 %) or with wheat flour (30 %) is used as staple food in Egypt	Galal (2001)
Fenugreek contains 23-26 % protein, 6-7 % fat and 58 % carbohydrate and ~25 % dietary fiber.	USDA (2001)
Properties of fenugreek mucilage and its use in ice cream as a stabilizer	Balyan <i>et al.</i> (2001)
Nutritional value and physiological properties of fenugreek.	Billaud and Adrian (2001)
Improvement of oxidative stability of raw food products like eggs by fenugreek	Armitage <i>et al.</i> (2002)
Fenugreek seed mucilages can be used as viscosity builders	Seghal <i>et al.</i> (2002)
Supplementation of fenugreek flour in rice bran improved the physical and sensory properties and qualities of breads and cookies	Sharma and Chauhan (2002)
Reduction in phytic acid levels and simultaneous <i>in vitro</i> increase with respect to calcium and iron with higher temperature and longer fermentation duration in case of fenugreek supplemented Indian bread	Bhatia and Khetrapaul (2002)
Analysed the composition of raw, soaked and germinated seed and found that nutritional quality of fenugreek seed could be improved with subsequent reduction of bitterness through processing	Hooda and Jood (2003a.)
Physiological, rheological and organoleptic characteristics of wheatfenugreek supplemented blends showed increase in protein and fat contents	Hooda and Jood (2003b.)

Source:- seed production technology for fenugreek (Saikat , 2006)



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Figure 2.6 Fenugreek seeds, ungrounded and grounded

2.7.3 Nutraceutical properties of fenugreek

Fenugreek has been referred to as a medicinal herb both in Indian Ayurvedic and traditional Chinese medicines (Tiran, 2003). Ancient literature, religious scripture, travel records and anecdotes from different continents and from different periods of human history, record a wide variety of medicinal properties associated with fenugreek (Lust, 1986). Medicinal uses vary from wound-healing to bust enhancement and, from promotion of lactation in weaning mothers, to its use as a sex stimulant or aphrodisiac

(Petropoulos 2002; Tiran 2003). Although Fugh-Berman (2003) has suggested that there is little clinical evidence to support these claims, fenugreek leaves and seed have been used extensively to prepare extracts and powders for medicinal use (Basch *et al.*, 2003). A number of important chemicals with medicinal value have been found in fenugreek seed and leaves (Petropoulos, 2002). Some of these are presented in Table 2.5.

Table 2.5 Fenugreek and its associated medicinal properties.

Medicinal properties reported	References
Anti-diabetic and cholesterol lowering proper ties	Devi <i>et al.</i> (2003)
Anti- hyperthyroidism	Tahiliani and Kar (2003a)
Against thyroxine-induced hyperglycaemia	Tahiliani and Kar (2003b)
Anti-cancer effects	Devasena and Menon (2003)
Gastro-protective effect	Pandian <i>et al.</i> (2002)
Antioxidant property	Raskin <i>et al.</i> (2002)
Antinociceptive property	Javan <i>etal.</i> (1997)
Antimicrobial property	Bhatti <i>etal.</i> (1996)
Anthelmintic property	Ghafgazi <i>et al.</i> (1980)
Anti-sterility and anti-androgenic effects	<i>Kamal et al.</i> (1993)
Wound healing property	Taranalli and Kuppast (1996)
Anti-inflammatory and antipyretic actions	Ahmadiani <i>et al.</i> (2001)

Source: - seed production technology for fenugreek (Saikat , 2006)

In general, fenugreek contains three important chemical constituents with medicinal value; *i.e.* 1) steroidal sapogenins; 2) galactomannans and 3) isoleucine. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products (Srichamroen *et al.*, 2005). Some medicinal properties attributed to fenugreek are presented in Table 2.5.

Steroidal sapogenins

Fenugreek seed is an important source of steroidal sapogenins such as diosgenin which are used extensively by both pharmaceutical and nutraceutical industries (Srichamroen *et al.*, 2005). Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones

such as testosterone, glucocorticoids and progesterone (Fazli and Hardman, 1968; Raghuram *et al.*, 1994; Srichamroen *et al.*, 2005). McAnuff *et al.*, (2002) reported that steroidal sapogenins are effective agents for the treatment of hypocholesterolemia, a disorder often associated with diabetes. At present natural diosgenin is procured chiefly from the tubers of certain wild species of Mexican yam (*Dioscorea* species). However this process is both time consuming and costly, requiring several years before the yam tubers grow to a size where they possess a sufficient concentration of diosgenin to be used as source of commercial and pharmaceutical reagent (Rosser, 1985). Fenugreek may be a viable alternative for production of diosgenin because of its shorter growing cycle, lower production costs and, consistent yield and quality (Hardman, 1969; Petropoulos, 1973).

Galactomanans

Galactomannans are a major polysaccharide species found in fenugreek seeds and, represent - 50% of the seed weight (Raghuram *et al.*, 1994). They make up an integral component of the cell walls in the seed endosperm (Meier and Reid, 1977). Their structure is composed of a 1-4 linked J3-D-mannosyl backbone with single unit galactose side-chains, a-linked at the 0-6 oxygen. Fenugreek galactomanan is considered unique due to a 1:1 to 1.2:1 ratio of galactose to mannose (G:M) molecules (Andrews *et al.*, 1952; Petropoulos, 2002). The high ratio of galactose substitution contributes to the hydrophilic properties associated with the galactomannans in fenugreek seed and, is important in determining the overall biological value of the galactomannans in fenugreek (Srichamroen *et al.*, 2005). Fenugreek galactomannans appear to be beneficial for control of type 2 diabetes in both animals (Puri *et al.*, 2002 ;) and humans (Madar *et al.*, 1988 ;) by reducing hyperglycemia in these individuals. They have a high water-binding capacity and are able to form highly viscous solutions at relatively low concentrations,

which appear to reduce glucose absorption in the digestive tract (Raghuram *et al.*, 1994). According to Ramesh *et al.* (2001) fenugreek gum (galactomanan) is under-exploited in our global food industry.

Isoleucine

The amino acid isoleucine is a precursor of 4-hydroxyisoleucine which is known to regulate the secretion of insulin in animals (Broca *et al.*, 2000; Sauvaire *et al.*, 1998). Most hypoglycaemic and anti-hyperglycaemic effects of fenugreek are attributed to the gastrointestinal effect of dietary fiber and systemic effects of amino acids like 4-hydroxyisoleucine present in the seed (Madar, 1984).

Table2.6 Major chemical constituents of fenugreek seed and leaves.

Broad group of Chemicals	Name of individual constituent Members	References
Major polysaccharides	Galactomannans (consisting of galactose and mannose in the ratio of 1:1)	Petropoulos (2002)
Major steroidal Sapogenins	Diosgenin[(25 R)-spirost-5-ene-3p-ol](Figure 2.5.1. F); yamogenin, tigogenin (Figure 2.5.1. E), neotigogenin, smilagenin and sarsasapogenin	Fazli and Hardman (1968); Sauvaire <i>et al.</i> (1991); Taylor <i>et al.</i> (1997)
Dihydroxy steroidal sapogenins (minor sapogenins)	Yuccagenin, gitogenin and Neogitogenin	Taylor <i>et al.</i> (1997)
Spirostanol saponins	Graecunin-B, C, D, E and G	Varshney and Jain (1979)
Triterpenoids		Shang <i>etal.</i> (1998)
N-compound	Trigonelline (methylbetaine derivative of nicotinic acid) (Figure 2.5.1. D)	Petropoulos (2002)
Flavonoids	Kaempferol, afroside, quercetin, isoquercitrin, vitexin, isovitexin, orientin, and luteolin	Huang and Liang (2000); Petropoulos (2002)
Isoflavonoid Phytoalexins	Medicarpin, maackiaian, vestitol and sativan	Petropoulos (2002)
Kaempferol glycosides	Lilyn	Han <i>et al.</i> (2001); Petropoulos (2002)
Phenolic compounds	Scopoletin (Figure 2.5.1. C), chlorogenic, caeffic acids, pcoumaric acids, hymercromone, coumarin (Figure 2.5.1. B) and trigocoumarin	Petropoulos (2002).
Stercal sapogeninpeptide Ester	Fenugreekine	Ghosalef a/. (1974).

Source:-seed production technology for fenugreek (Saikat , 2006)

MATERIALS AND METHODS

3.1 Study site

Experiments were carried out in the Laboratory of the Food Science and Nutrition Program, at the Science Faculty, Addis Ababa University and Ethiopian Health and Nutrition Institute (EHNRI). Addis Ababa is the capital city of Ethiopia with the altitude of 2444 m above sea level with a mean annual rainfall of 1196mm and located 9°01' N and 38°45' E. The minimum and maximum temperatures were 9.9°C and 24.6°C, respectively (Fissha & Olsson, 2004).

3.2 Procurement of materials

The bulk samples of commonly distributed and consumed varieties of *Tef*, namely, Quncho (very white seeded) and fenugreek, Chala were obtained from Debre Zeit Agricultural Research Center (DZARC). All the grains were cleaned, made free of dust and other foreign materials. The grain sample of *Tef* was milled into flour using a commercial mill and the fenugreek seeds were ground to fine powder with a mill (model no.: FW 100, all-purpose high-speed smashing machine -Electrical Grinder) to pass through a 0.425 mm sieve, and then stored in plastic containers for analytic use.

3.3 Processing methods

3.3.1. Soaking

Fenugreek seeds, cleaned and freed of broken seeds, dust and other foreign materials, were soaked in tap water for 12 h at 37 °C. A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice in distilled water and then dried at 55-60°C.

3.3.2. Germination

The soaked seeds were covered with wet clean cloth and placed in a clean basket for 48 h at 37°C, with frequent watering (2-3 times a day) to enhance the germination process. The sprouts were rinsed in distilled water and dried at 55-60°C.

3.3.3. Roasting

In dry heat treatments, whole grains were heated in a hot air oven at 110°C for an hour.

The dried samples of raw, roasted, soaked and germinated seeds were ground to fine powder using All-purpose high-speed smashing machine(FW 100 model)) and used for analysis.

3.4. Preparation of blends

Each treated (Roasted, soaked and germinated) and untreated (raw) fenugreek seed powder were blended separately with tef flour at four different levels namely, 5%, 10%, 15% and 20% as illustrated below in Table 3.1.

Table 3.1 Blend proportion

Ingredients	Blend proportions				
	0%	5%	10%	15%	20%
Tef:raw fenugreek	100:0	95:5	90:10	85:15	80:20
Tef:roasted fenugreek	100:0	95:5	90:10	85:15	80:20
Tef:soaked fenugreek	100:0	95:5	90:10	85:15	80:20
Tef:germinated fenugreek	100:0	95:5	90:10	85:15	80:20

3.5 Preparation of injera

Preparation of injera was carried out using pure *Tef* flour sample as control and blends of fenugreek seed flours. One control (whole *Tef*) and sixteen fenugreek blended injera were baked according to the traditional practices in Ethiopia where, the flour was fermented for three days then made into injera, sourdough type flat bread. The overall procedures followed to prepare injera were summarized in Fig 3.1.

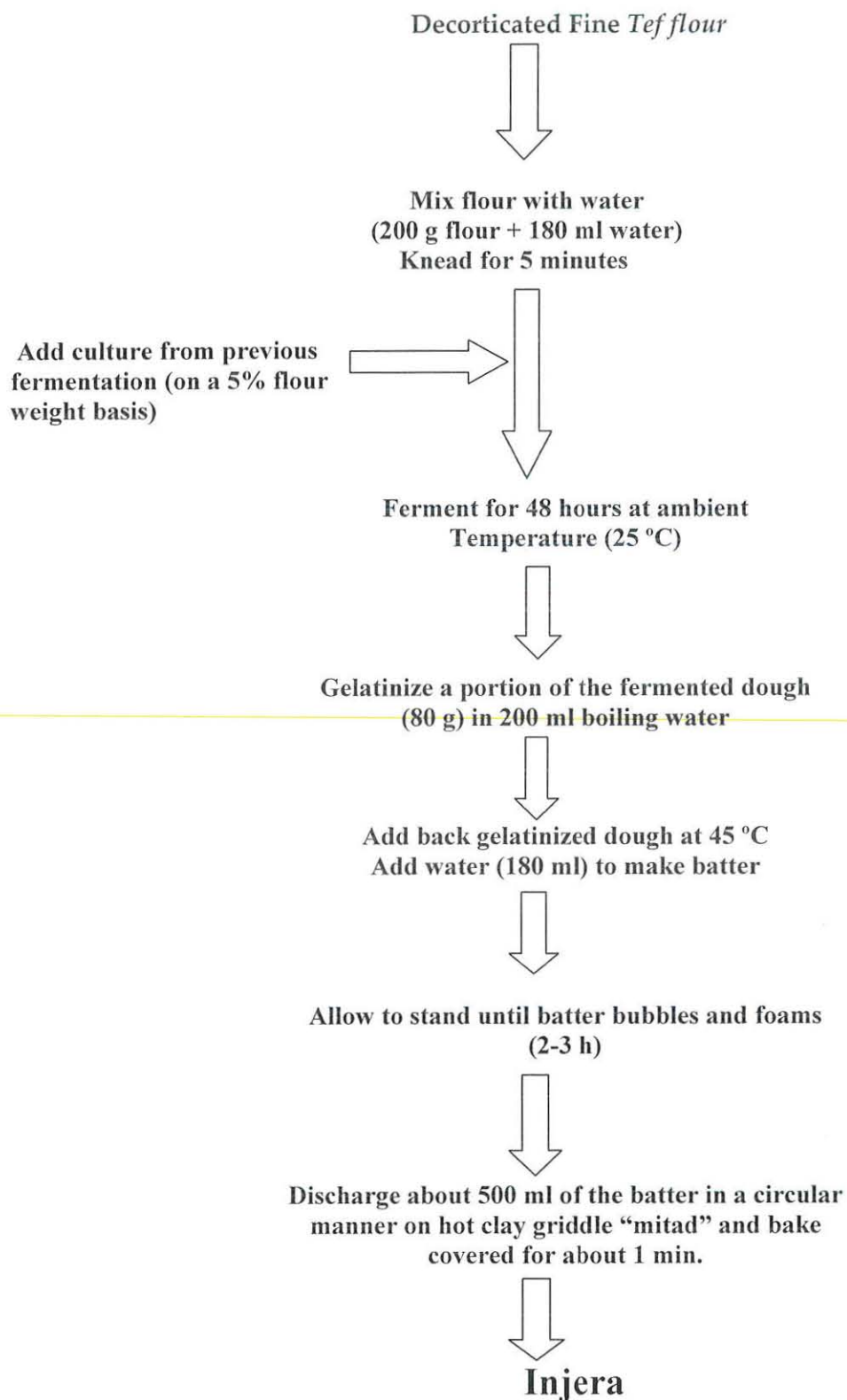


Figure 3.1 Flow chart for injera making

3.6 Proximate composition determination

Proximate chemical analyses of all the samples were done according to AOAC (2000), and total carbohydrate was calculated by difference. Brief description of methods is given in the following sub sections.

3.6.1 Determination of moisture content

Moisture of the seed flours and injera were determined according to AOAC (2000) using the official method 925.09. A crucible was dried in an oven at 105°C for 1 h and placed in desiccators to cool. The weight of the crucible (W_1) was determined. 5.0000g Sample was weighed in the dry crucible (W_2) dried at 105°C for 3 h and after cooling in desiccators to room temperature it was again weighed (W_3). The moisture content was determined using Eq. (1).

$$\text{Moisture content in percent (\%)} = \left(\frac{W_2 - W_3}{W_2 - W_1} \right) \times 100 \quad (1)$$

3.6.2 Crude protein

Protein content was determined according to AOAC (2000) using the official method 979.09.

Digestion: About 0.5000 g of fresh samples was taken in a Tecator tube and 6ml of concentrated sulfuric acid (AR) was added and mixed, and 3.5 ml of 30% hydrogen peroxide was added step by step. As soon as the violent reaction had ceased, the tubes were shaken and placed back to the rack. Three gram of catalyst (a mixture of copper sulphate pent hydrate and potassium sulphate) was added and left for 15 min. The mixture was digested in a digest stove (HYP-1008 eight holes) at 370°C for 4h. The digestion was continued until a clear solution was obtained .The tubes in the rack were cooled in a fume hood; 25ml of distilled water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation and titration: The digested and diluted sample solution was distilled using boric acid and the distillate was titrated using 0.1N Hydrochloric acid until reddish color is appeared (T. Manual, 1979). The crude protein was determined using Eq. (2).

$$\text{Nitrogen (\%)} = \frac{V_{HCL} \text{ in L} \times N_{HCL} (ca. 0.1) \times 14.00 \times 100}{W_o}$$

$$\text{Protein (\%)} = 6.25 \times \% \text{ Nitrogen} \quad (2)$$

Where:

V is volume of HCl in L consumed to the end point of titration,

N is the normality of HCl (used often is 0.1N),

W_o is sample weight on dry matter basis and 14.00 is the molecular weight of nitrogen.

The % of nitrogen is converted to % of protein by using appropriate conversion factor (% protein = 6.25 x % N for both the tef and fenugreek).

3.6.3 Determination of crude fat

The crude fat was extracted according to AOAC (2000) official method 4.5.01. The cleaned flask (cylinder) and boiling chips were dried in the drying oven at 100⁰C for 1h, cooled in the desiccators for 30min and weighed (W₁). Two grams of sample was weighed (W) in thimble containing fat free cotton. The thimbles were placed in the thimble holders, 50ml of Diethyl ether was poured in the flask, the thimble was immersed in the solvent (in the flask) and heated at 55⁰C in the fat determinator (SZC-C fat determinator) for 1h, hanged the thimble and heated at the same temperature for 2hs and then the solvent was recovered for 15 min. The heater was switched off, the flask was dried in the drying oven at 70⁰C for 30 min, cooled in the desiccators for 15 min and then weighed the flask with the extract (W₂). The crude fat was determined using Eq. (3).

$$\text{Crude fat, percent by weight} = \left(\frac{W_2 - W_1}{W} \right) \times 100 \quad (3)$$

Where: W_1 = weight of the extraction flask (g)

W_2 = weight of the extraction flask plus the dried crude fat (g)

W = weight of sample (g)

3.6.4 Determination of ash content

The ash content was determined by AOAC (2000) using the official method 923.03. The cleaned crucibles were dried in a muffle furnace at 550°C for 1h and cooled in desiccators for 30 min and weighed to the nearest milligram (M_1). About 2.5000 g of fresh sample was placed in dish and weighed (M_2). Dishes were placed on a hot plate under a fume-hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes with sample were placed inside the muffle furnace at 550°C for 5 hs and cooled in desiccators for 1 h. The ash was clean and white in appearance. When cooled to room temperature, each dish with ash was reweighed to the nearest milligram (M_3). The Ash content was determined using Eq. (4).

$$\text{Total Ash (\%)} = \left(\frac{M_3 - M_1}{M_2 - M_1} \right) \times 100 \quad (4)$$

Where: ($M_2 - M_1$) is sample mass in g on dry base and ($M_3 - M_1$) mass of ash in g

3.6.5 Determination of crude fiber

Crude fiber analysis was conducted using the method of AOAC (2000) official method.

Digestion: About 1.7000 g of fresh sample (W_3) was placed into a 600 ml beaker; 200 ml of 1.25% H_2SO_4 was added, and boiled gently for 30 minutes while watch glass was placed over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After exactly 30 min heating, 20 ml of 28% KOH was added and boiled gently for further 30 min, with occasional stirring.

Filtration: The bottom of a sintered glass crucible was covered with 10mm sand layer and wetted with distilled water. The solution was poured into sintered glass crucible and filtered with the aid of vacuum pump (High Performance Vacuum Pump, Robin Air way, SPX Corporation, Montplier, USA). The wall of the beaker was rinsed with hot distilled water several times; washings was transferred to the crucible and filtered.

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

Drying and combustion: The crucible with its content was dried in a drying oven for 2 h at $130^{\circ}C$ and cooled for 30 min in a desiccators (with granular silica gel), and then weighed (W_1). The crucible was transferred to muffle furnace and heated for 30 min at $550^{\circ}C$. The crucible was cooled in desiccators and weighed (W_2). The crude fiber was determined using Eq. (5).

$$\text{Crude fiber g/100g} = [(W_1 - W_2) \times 100] / W_3 \quad (5)$$

Where: - W_1 = weight of crucible with sample after drying

W_2 = weight of crucible with sample after ashing

W_3 = fresh sample weight

3.6.6 Determination of crude carbohydrate:- The crude carbohydrate was calculated by difference. The mathematical expression is as Follow (eq.6).

$$\%C = 100 - [\%M + \%P + \%F + \%Fb + \%A] \quad (6)$$

Where:

C-Carbohydrate content, M-Moisture content, P- crude Protein content, F- crude Fat content, Fb- crude Fiber content and A-Ash content.

3.6.7 Gross energy (in Kcal)

Gross energy was calculated according to Osborne and Voogt (1978):

The gross energy content of the sample was calculated as follow:

$$\text{Gross energy (\%)} = (9 \times \text{crude fat \%}) + (4 \times \text{crude protein \%}) + (4 \times \text{crude carbohydrate \%}) \quad (7)$$

3.7 Determination of minerals

Calcium, zinc and iron were determined using atomic absorption spectrophotometer method of Osborne and Voogt (1978). The ash obtained after dry ashing at 550°C was treated with 5 ml of 6N HCl to wet it completely and carefully dried on a low temperature hot plate. 7 ml of 3N HCl was added and the dish was heated on the hot plate until the solution just boils. Then, it has been cooled and filtered through a filter paper in to a 50ml volumetric flask. Again 7 ml of 3N HCl was added to the dish and heated until the solution just boils. Finally, cooled and filtered into the volumetric flask. For the determination of calcium, lanthanum chloride (1% w/v) was added to

both standards and samples to suppress interference from phosphorus. Using atomic absorption spectrophotometer (Varian, spectra-10/20, Australia) a calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100g. Reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption or emission values of the samples and the blank. The metal contents were calculated by using the formula eq.8

$$\text{Metal content (mg/100g)} = \frac{[(A-B) \times V]}{10W} \quad (8)$$

Where,

W = weight of samples (g)

V = volume of extract (ml)

A = concentration of sample solution (µg/ml)

B = concentration of blank solution (µg/ml)

3.8 Determination of anti-nutritional factors

3.8.1 Determination of phytic acid

Phytic acid was determined by using Latta and Eskin (1980) as modified by Vaintraub and Lapteva (1988). About 1g of sample was extracted with 10 ml 2.4% HCl for 1h at an ambient temperature and centrifuged (3000 rpm/30min) (Nüve, bench-top centrifuge, NF 800R, 2001, Ankara, Turk). The supernatant was used for phytate estimation. About 2 ml of Wade reagent (0.03% solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ containing 0.3% sulfosalicylic acid in water) was added to 3 ml of the sample solution and centrifuged. The absorbance at 500 nm was read using spectrophotometer (BECKMAN, DU-64, Japan). The phytate concentration was calculated from the difference between the absorbance of the control (3ml of water + 1ml of Wade reagent) and that of assayed sample. The concentration of phytate was calculated using phytic acid standard curve (by preparing a series of standard solution containing 3.9, 7.8, 15.5, 23.4, 31.2 and 39

µg/ml phytic acid in 0.2 N HCl) using water to zero the spectrophotometer, and results were expressed as of phytic acids in mg/100gm dry weight by the following formula.

$$\text{Phytic acid (ug/g)} = \frac{\text{Absorb-Intercept}}{\text{Slope} \times \text{Density} \times \text{wt.sample}} \times \frac{10}{3} \quad (10)$$

Where, Abso= Absorbance
Density= density of the solvent

3.8.2 Determination of tannin

The amount of condensed tannin was determined by the Vanillin assay of Burns (1971) as modified by Maxson and Rooney (1972). About 1g of sample was weighed, extracted with 10ml 1% HCl in methanol, at room temperature in mechanical shaker for 24 h. The mixture was centrifuge at 1000G for 10 min. 1ml supernatant was mixed with 5ml of vanillin- HCl reagent in another test tube. When the reaction was completed (after 20 min), the absorbance was read at 500nm using spectrophotometer (BECKMAN, DU-64, Japan). Dcatechen was used as standard value of tannin in mg D-catechen per gram of sample. 40mg D-catechen was dissolved in 100ml of 1%HCl in methanol and from this 0, 0.2, 0.4, 0.6, 0.8 and 1ml was taken in a test tube and the volume was adjusted to 1ml with 1% HCl in methanol. 5ml of vanillin-HCl reagent in each test tube was added. After 20 min the absorbance was read at 500nm. The absorbance of the blank was subtracted from the absorbance of the corresponding vanillin-containing sample. A standard curve has been constructed (Absorbance vs. D-catechin) and the linear portion of the curve was extrapolated to produce the standard curve. Finally, the tannin contents were calculated. Values of tannins were expressed in milligram of D-catechin equivalent per gram of sample by the following formula.

$$\text{Tannin (mg/100g)} = \frac{\text{Absorb-Intercept}}{\text{Slope} \times \text{Density} \times \text{wt.sample}} \times (\text{Dilution factor})$$

3.9 Sensory evaluation

The organoleptic characteristics of injera made from blended *Tef* flours (were evaluated using a taste panel, consisting of 10 judge. The panelists were selected from the staff and students of Food Science and Nutrition program.

The panelists were instructed about the purpose and objective of the test, so the selection of panelists were based on basic requirements of a panelist, such as availability for the entire period of evaluation, interest and willing to serve. The health status of the panelists was also considered during panelist selection (not suffering from colds and allergy that affect their sensitivity to the product).

Panelists were asked to rinse their mouth with tap water that was provided to them, before the next serving. The containers with the samples were coded.

The panelists were asked to rank the injera on the basis of appearance, color, taste, flavor, odor and texture using a nine point hedonic scale, (where 1 = dislike extremely and 9 = like extremely). Overall acceptability of the samples were also rated on the same scale with 9 = extremely acceptable and 1 = extremely unacceptable (Inyang and Idoko, 2006).

The sensory evaluation was conducted between 3:00 and 4:00 pm, during which panelists were neither too satisfied nor too hungry, assuming that they had their lunch.

3.10 Data analysis

The effect of blend proportion and processing methods on the quality of the product were analyzed with two factors ANOVA. When P values were found significant ($P < 0.05$), the means of each parameter were compared using the least significant differences (LSD) procedures of the SPSS, version 16 for windows. And also multiple comparison procedure of the treatment means was performed by Duncan's new multiple range tests.

RESULT AND DISCUSSION

4.1. Proximate analysis results of treated, untreated fenugreek and tef flour

4.1.1 Moisture content

In the present study, mean moisture content of the treatments ranged from 6.4-8.16g/100g respectively for roasted fenugreek and germinated fenugreek (Table 4.1.1). The mean moisture content of the raw fenugreek was (7.45 g/100g), which is in agreement with the finding of EHNRI (1998). The moisture content of germinated fenugreek flour gave significantly ($p<0.05$) higher mean followed by raw fenugreek which also significantly differed from roasted fenugreek. However, moisture content of the raw and soaked flour samples varied slightly significantly ($P>0.05$). All the treatments except raw grains were subjected to drying operation in order to prepare flour for later use. This drying resulted in the flours with moisture content even less than the original raw flour. The relative increment of moisture content of germinated fenugreek sample may be attributed to a variation in the treatment during the drying process of the samples.

Table 4.1.1 proximate analysis results of treated, untreated fenugreek and tef flour (On dry matter basis)

Treatments	Moisture (g/100g)	Protein (g/100g)	Fat (g/100g)	Crude Fiber (g/100g)	Total ash (g/100g)	CHO (g/100g)	Energy (Kcal/100 g)
Tef	10.96±0.07 ^a	9.19±0.06 ^c	3.09±0.01 ^d	4.89±0.06 ^c	2.23±0.01 ^c	69.66±0.08 ^a	343.15±0.15 ^d
RwF	7.45±0.10 ^{cd}	30.55±0.45 ^b	8.33±0.47 ^c	9.08±0.02 ^b	3.33±0.16 ^a	41.27±0.68 ^b	362.26±3.33 ^b
RaF	6.40±0.36 ^d	29.90±0.12 ^b	10.85±0.02 ^a	9.12± 0.03 ^b	3.29±0.05 ^a	35.42±0.40 ^d	358.98±0.97 ^{bc}
SF	7.03±0.14 ^{cd}	30.40±0.28 ^b	8.52±0.01 ^c	9.54±0.03 ^b	2.67±0.04 ^b	39.31±0.41 ^c	355.53±0.59 ^c
GF	8.16±0.15 ^b	31.41±0.41 ^a	9.53±0.30 ^b	12.07±0.04 ^a	2.11±0.13 ^c	39.26±0.94 ^c	368.47±1.44 ^a

Any two means in the same column not followed by the same letters are significantly different ($P<0.05$).

NB: T-tef, RwF-raw fenugreek, RaF-roasted fenugreek, SF-soaked fenugreek, GF-germinated fenugreek and values are Mean±SE

4.1.2 Crude protein

The mean crude protein content of the treatments ranged from 29.90-31.41g/100g for roasted fenugreek and germinated fenugreek respectively (Table 4.1.1). Crude protein content of raw fenugreek was (30.55g/100g), which is in close agreement with the finding of EHNRI (1998) that is (28.90 g/100g). Robert (1994) also reported that the raw fenugreek contained 30 g/100g crude protein. The crude protein content of germinated fenugreek flour gave significantly ($p<0.05$) higher mean than that of raw, roasted and soaked fenugreek. However, moisture content of the raw, roasted and soaked flour samples varied slightly significantly ($P>0.05$). The results obtained were in agreement with those obtained with kidney bean (Alonso *et al.*, 2000) and mungbean (Mubarek, 2005). Germination has been shown to increase protein content of legumes (Kyllen and McCready, 1975; Fordham *et al.*, 1975). The increase in protein during germination was due to the release of free amino acids after enzymatic hydrolysis for the synthesis of new protein (Bliss, 1975). Morgan *et al.*, (1992) found that the protein content of sprouts increased from the time of germination, the absorption of nitrates facilitates the metabolism of nitrogenous compounds from carbohydrate reserves, thus increasing crude protein levels. However roasting and soaking of fenugreek caused slight reduction in crude protein level. The crude protein content during roasting and soaking was found to be 29.90 % and 30.40%, respectively. Loss of crude protein in heat treated grain could be due to the denaturation and degradation of protein. Reduction in crude protein content with respect to soaking treatments might be due to the leaching out of low molecular weight nitrogen compounds in to the leachate as suggested by Sharma and Kapoor (1997).

4.1.3 Crude fat

Effect of treatments on fat content of fenugreek flour is presented in Tables 4.1.1. It ranged from 8.33-10.85g/100g for raw and roasted fenugreek respectively (Table 4.1.1). The fat content of raw fenugreek was (8.33 g/100g), which is in close agreement with the finding of EHNRI (1998)

(7.2g/100g). The fat content of roasted fenugreek flour gave significantly ($p<0.05$) higher mean followed by germinated fenugreek which also significantly differed from untreated fenugreek, and soaked fenugreek. However, moisture content of the raw and soaked flour samples varied slightly significantly ($P>0.05$). Change in the fat content by roasting and germination were significant ($P<0.05$). Similar result was reported on African locust bean by Esenwah and Ikenebomeh (2008). The increased result might be due to roasting and germination of the sample that led to the cleavage of the protein-lipid or carbohydrate-lipid linkages, there by facilitating the easy extraction of the oil by the extracting solvent (Esenwah and Ikenebomeh, 2008). Soaking processes have negligible effect on crude fat content of the legume flours.

4.1.4 Crude fibre

The result of of treatments effects on the crude fibre of fenugreek is shown in Tables 4.1.1. It was ranged from 9.08-12.07g/100g for raw and germinated fenugreek respectively. The crude fibre content of germinated fenugreek flour gave significantly ($p<0.05$) higher mean than that of raw, roasted and soaked fenugreek. There was no significant change in all the treatments except for germination. It may be attributed to the fact that germination stimulates the synthesis of more cell wall materials to support the roots thereby increases the fibre contents (Akubar and Obiegbuna, 1999). In soaking and roasting treatments there was no change in the amount of insoluble and non digestible materials and therefore there was no significant variation in terms of crude fibre content (Milik *et al.*, 2002).

4.1.5 Total ash content

Effect of treatments on ash content of fenugreek flour is presented in Tables 4.1.1. It ranged from 2.11-3.33(g/100g) for germinated and raw fenugreek respectively. The total ash content of raw fenugreek was 3.33 g/100g, which is in agreement with the finding of EHNRI (1998) (3.6g/100g). The ash content of raw fenugreek flour gave significantly ($p<0.05$) higher mean followed by

roasted fenugreek which also significantly differed from soaked and germinated fenugreek. However, ash content of the raw and roasted fenugreek flour samples varied slightly significantly ($P>0.05$). The result showed that ash content of the soaked and germinated samples was 2.67% and 2.11% respectively. It is lower than the raw and roasted flours 3.33% and 3.29% respectively. The leaching of minerals from the seeds during soaking could be the reason. It was observed that the legumes contained a large portion of water soluble ash which has the tendency to leach out during hydro processing of seeds (Esenwah and Ikenebomeh, 2008). Loss in ash might be due to leaching of soluble inorganic salts into the processing water during the soaking of the sample (Esenwah and Ikenebomeh, 2008).

4.1.6 Crude carbohydrate

The mean crude carbohydrate content of the treatments ranged from 35.42-41.27g/100g for roasted fenugreek and raw fenugreek respectively (Table 4.1.1). The present finding is consistent with the finding of Hooda and Jood (2002) who reported the range to be from 43.74 to 38.83. The carbohydrate content of raw fenugreek flour gave significantly ($p<0.05$) higher mean followed by soaked and germinated fenugreek which significantly differed from roasted fenugreek. However, carbohydrate content of the soaked and germinated flour samples varied with slight significance ($P>0.05$). Loss in carbohydrate during soaking and then germinating might be due to leaching of soluble carbohydrates like sugars into the soaking water. The increase recorded in the crude protein and ether extract may be due to the reduction in the carbohydrate content and may be regarded as apparent increase in both the protein and fat contents to complement the decrease in carbohydrate Esenwah and Ikenebomeh (2008).

4.1.7 Gross energy

The result of treatments effects on gross energy of fenugreek is shown in Tables 4.1.1. It was ranged from 355.53-368.47 Kcal/100g for soaked and germinated fenugreek respectively

(Table 4.1.1). The mean gross energy content of raw fenugreek was 362.26 Kcal/100g, which is in agreement with the finding of EHNRI (1998) (360 Kcal/100g). The Gross energy of germinated fenugreek flour gave significantly ($p < 0.05$) higher mean followed by raw fenugreek which significantly differed from soaked fenugreek. There was no significant difference ($P > 0.05$) in the gross energy content of roasted and raw fenugreek.

The proximate composition of tef flour is given in table 4.1.1. The moisture, protein, fat and ash contents of tef flour were found to be 10.96, 9.19, 3.09 and 2.23 (g/100g) respectively. These are in agreement with the finding of EHNRI (1998) (10.90, 9.30, 2.4 and 2.4 g/100g) respectively. However, EHNRI reported much lesser fiber values (2g/100g). The variations in fiber content may be attributed to the variety differences.

According to the results in Table 4.1.1, tef flour compared to fenugreek flour was found to have lower in all chemical compositions except moisture content, which agrees with the finding of Bahnassey *et al.*, (1986). Comparison of chemical composition of legume flours and their concentrates with semolina showed that all legume flours contained significantly higher protein, ash, fiber and fat contents than the semolina.

4.2 Sensory characteristics

The effects of fenugreek supplementation on the sensory characteristics of injera are presented in Tables 4.2.1, 4.2.2 and 4.2.3. With the increase in the level of fenugreek flour (raw, roasted, soaked and germinated) in formulation, the sensory scores for appearance, color, taste, odor, texture and overall acceptability of injera decreased sharply. This might be due to the bitter taste of fenugreek. Similar observation with supplementation of fenugreek (Hooda and Jood, 2005) with wheat flour was also reported.

Table 4.2.1 Effect of blending ratio on sensory characteristics of supplemented injera

Blend proportion	Appearance	color	Taste	Odor	Texture	Over all Accept.
(100%) <i>Tef</i>	8.30±0.10 ^a	8.30±0.07 ^a	8.50±0.08 ^a	8.50±0.08 ^a	8.40±0.11 ^a	8.40±0.08 ^a
5% FB	7.23±0.19 ^b	7.40±0.19 ^b	6.53±0.28 ^b	7.03±0.22 ^b	7.15±0.22 ^b	7.13±0.22 ^b
10% FB	4.75±0.34 ^c	5.23±0.31 ^c	4.30±0.29 ^c	4.75±0.31 ^c	4.70±0.32 ^c	4.78±0.29 ^c
15% FB	2.78±0.30 ^d	2.48±0.34 ^d	2.10±0.23 ^d	2.18±0.28 ^d	2.58±0.31 ^d	2.45±0.27 ^d
20% FB	2.20±0.21 ^e	1.93±0.25 ^e	1.78±0.19 ^d	1.83±0.25 ^d	2.18±0.26 ^d	2.05±0.20 ^d

Any two means in the same column not followed by the same letters are significantly different (P<0.05).

The sensory quality of injera (Appearance, color, taste, odor texture and over all acceptability) were slightly increase with the supplementation of treated (soaked and germinated) fenugreek than untreated (raw) fenugreek supplemented injera. However, the sensory quality of roasted fenugreek supplemented injera was decrease.

Table 4.2.2 Effect of processing on sensory characteristics of supplemented injera

Blend proportion	Appearance	color	Taste	Odor	Texture	Over all Accept.
RwFB	4.56±0.39 ^c	4.78±0.40 ^b	4.38±0.40 ^b	4.64±0.42 ^b	4.62±0.41 ^b	4.54±0.40 ^{bc}
RaFB	4.06±0.41 ^d	3.92±0.45 ^c	3.78±0.43 ^c	3.92±0.45 ^c	3.98±0.42 ^c	4.16±0.41 ^c
SFB	4.98±0.42 ^b	4.86±0.47 ^b	4.56±0.44 ^b	4.62±0.46 ^b	4.96±0.43 ^b	4.94±0.42 ^b
GFB	6.60±0.30 ^a	6.70±0.27 ^a	5.84±0.35 ^a	6.32±0.32 ^a	6.44±0.32 ^a	6.20±0.34 ^a

Any two means in the same column not followed by the same letters are significantly different (P<0.05).

As it can be seen from the above tables (4.2.1 and 4.2.2), sensory quality of the supplemented injera has been observed to be significantly affected by both processing and blend proportion. From the overall acceptability rating, it was concluded that fenugreek flour could be incorporated up to 5% level in all treated and untreated fenugreek flour and 10% level for soaked and germinated fenugreek flour in the formulation of injera without affecting their sensory quality.

Table 4.2. 3. Effect of processing*blending on sensory characteristics of supplemented injera

Treat &BP	appearance	color	taste	odor	texture	Overall accp.
control	8.3±0.29	8.3±0.28	8.5±0.36	8.6±0.34	8.4±0.36	8.4±0.34
T:RwF						
95:5	7.0±0.29	7.3±0.28	5.7±0.36	6.8±0.34	7.3±0.36	7.5±0.34
90:10	3.5±0.29	4.7±0.28	2.3±0.36	4.4±0.34	3.8±0.36	7.0±0.34
85:15	2.0±0.29	2.0±0.28	1.2±0.36	2.0±0.34	1.9±0.36	5.3±0.34
80:20	2.0±0.29	1.6±0.28	1.2±0.36	1.4±0.34	1.7±0.36	4.0±0.34
T:RaF						
95:5	6.3±0.29	6.5±0.28	5.7±0.36	6.2±0.34	6.0±0.36	6.1±0.34
90:10	2.5±0.29	2.8±0.28	2.3±0.36	2.8±0.34	2.5±0.36	3.1±0.34
85:15	1.6±0.29	1.0±0.28	1.2±0.36	1.0±0.34	1.5±0.36	1.6±0.34
80:20	1.6±0.29	1.0±0.28	1.2±0.36	1.0±0.34	1.5±0.36	1.6±0.34
T:SF						
95:5	7.7±0.29	8.0±0.28	6.6±0.36	7.5±0.34	7.8±0.36	7.6±0.34
90:10	5.6±0.29	6.0±0.28	5.3±0.36	5.0±0.34	5.5±0.36	5.5±0.34
85:15	1.7±0.29	1.0±0.28	1.2±0.36	1.0±0.34	1.6±0.36	1.6±0.34
80:20	1.6±0.29	1.0±0.28	1.2±0.36	1.0±0.34	1.5±0.36	1.6±0.34
T:GF						
95:5	7.9±0.29	7.8±0.28	7.6±0.36	7.6±0.34	7.5±0.36	7.9±0.34
90:10	7.4±0.29	7.4±0.28	6.1±0.36	6.8±0.34	7.0±0.36	6.7±0.34
85:15	5.8±0.29	5.9±0.28	4.0±0.36	4.7±0.34	5.3±0.36	4.6±0.34
80:20	3.6±0.29	4.1±0.28	3.0±0.36	3.9±0.34	4.0±0.36	3.4±0.34

As it can be seen from table 4.2.3, the control samples had maximum overall acceptability, whereas injera containing 15% and 20% soaked and germinated fenugreek flours, injera contains 10%, 15% and 20% raw and roasted fenugreek flour were found to be unacceptable to the panelists. The overall acceptability score for control was 8.40 on a 9-point hedonic scale. Injera made from blends containing 5% level of all fenugreek flours were acceptable. At 15% and 20% levels of substitution, the overall acceptability was rated as poor. The germinated fenugreek flour-supplemented injera performed better than the other fenugreek-supplemented injera followed by soaked fenugreek flour supplemented injera. Similar observations with supplementation of soy flour (Dhingra & Jood, 2001; Singh *et al.*, 1996), bengal gram flour (Patel & Rao, 1996) and wheat bran (Sharma & Chauhan, 2002) with wheat flour were also reported.

4.3 Proximate analysis of control and supplemented injera

4.3.1 Moisture content

In the present study, mean moisture content of the blended injera ranged from 6.32-6.78g/100g respectively with levels of control and 10% blends (Table 4.3.1). The moisture content of 10% blended injera gave significantly ($p<0.05$) higher mean followed by 5% blended injera which also significantly differed from control injera. The present finding is consistent with the finding of Shalini and Sudesh (2005), also reported that the slight increase in moisture content of fenugreek fortified biscuits. It might be due to the higher water binding capacity of fenugreek flour used. Moisture content of the treatments ranged from 6.21-7.02 (g/100g) respectively for germinated and soaked fenugreek supplemented injera (Table 4.3.2). The moisture content of soaked fenugreek supplemented injera gave significantly ($p<0.05$) higher mean followed by raw fenugreek supplemented injera which also significantly differed from roasted and germinated fenugreek supplemented injera. The relative difference of moisture content may be attributed due to a variation in the treatment during the drying process of the samples.

Table 4.3.1 Effect of blending ratio on proximate composition of injera

Blend Prop.	Moisture (g/100g)	Protein (g/100g)	Fat (g/100g)	Fiber (g/100g)	Total ash (g/100g)	CHO (g/100g)	Energy (Kcal/100 g)
100% <i>Tef</i>	6.32±0.02 ^c	9.08±0.13 ^c	3.26±0.03 ^b	1.61±0.03 ^c	2.57±0.00 ^a	77.15±0.12 ^a	375.26±0.09 ^a
5% FB	6.63±0.14 ^b	9.43±0.11 ^b	3.73±0.17 ^b	2.94±0.23 ^b	2.50±0.03 ^b	74.80±0.20 ^b	370.45±1.82 ^b
10% FB	6.78±0.33 ^a	10.42±0.22 ^a	3.65±0.25 ^a	3.20±0.20 ^a	2.48±0.03 ^b	73.57±0.60 ^c	368.78±2.03 ^c

Any two means in the same column not followed by the same letters are significantly different ($P<0.05$).

4.3.2 Protein

The mean protein content of the blended injera ranged from 9.08-10.42(g/100g) respectively with levels of control and 10% blends (Table 4.3.1). The protein content of 10% blended injera gave

significantly ($p < 0.05$) higher mean followed by 5% blended injera which also significantly differed from control injera. Similarly Sharma and Chauhan (2000) also reported significantly ($P < 0.05$) higher protein content of breads prepared from blends of wheat-fenugreek flours. The increase in protein content of fenugreek supplemented injera might be the result of the appreciably higher protein content of fenugreek.

Protein content of the treatments ranged from 9.28-10.07 (g/100g) respectively for roasted and germinated fenugreek supplemented injera (Table 4.3.2). The crude protein content of germinated fenugreek supplemented injera gave significantly ($p < 0.05$) higher mean than raw, roasted and soaked fenugreek supplemented injera. However, protein content of the raw, roasted and soaked supplemented injera varied slightly significantly ($P > 0.05$). Germinated fenugreek supplemented injera contained higher amount of protein, other research workers also observed similar results in wheat and fenugreek composite flour Shalini and Sudesh (2004).

4.3.3 Fat content

The result of of blending ratio effects on fat content of fenugreek supplemented injera is shown in Tables 4.2.1. It ranged from 3.26-3.65(g/100g) respectively with levels of control and 10% blends (Table 4.3.1). The fat content of 10% blended injera gave significantly ($p < 0.05$) higher mean followed by 5% blended injera which also significantly differed from control injera. As the amounts of fenugreek flour in the formulation increases, the amount of fat in injera increase. This is may be due to the presence of high amount of fat in the fenugreek flour (8.33%) than is present in tef flour (3.09%).

Fat content of the treatments ranged from 3.14-3.87 (g/100g) respectively for soaked and roasted fenugreek supplemented injera (Table 4.3.2). The fat content of roasted fenugreek supplemented injera gave significantly ($p < 0.05$) higher mean followed by germinated fenugreek supplemented injera which also significantly differed from raw and germinated fenugreek supplemented injera.

However, fat content of the raw and soaked fenugreek supplemented injera varied slightly significantly ($P>0.05$). The increased result might be due to roasting and germinating of the sample that led to the cleavage of the protein-lipid or carbohydrate-lipid linkages thereby, facilitating the easy extraction of the oil by the extracting solvent (Esenwah and Ikenebomeh, 2008). Soaking processes have negligible effect on crude fat content of the legume flours (Gupta *et al.*, 1998).

4.3.4 Crude fibre content

The mean crude fibre content of the blended injera ranged from 1.61-3.20(g/100g) respectively with levels of control and 10% blends (Table 4.3.1). The crude fibre content of 10% blended injera gave significantly ($p<0.05$) higher mean followed by 5% blended injera which also significantly differed from control injera. As the amounts of fenugreek flour in the formulation increases, the amount of crude fibre in injera increase. This is may be due to the presence of high amount of fibre in the fenugreek flour (9.08%) than is present in tef flour (4.89%).

Crude fibre content of the treatments ranged from 2.70-2.96 (g/100g) respectively for raw and germinated fenugreek supplemented injera (Table 4.3.2). The crude fibre content of germinated fenugreek supplemented injera gave significantly ($p<0.05$) higher mean followed by roasted fenugreek supplemented injera which also significantly differed from raw fenugreek supplemented injera. However, fibre content of the germinated, roasted and soaked supplemented injera varied slightly significantly ($P>0.05$). This result is in agreement with the result reported by Shalini and Sudesh (2005), where the moisture, protein, fat and crude fibre contents increased with increasing (raw, soaked and germinated) fenugreek flour in wheat flour.

Table 4.4.2 Effect of processing on proximate composition of supplemented injera

Treatm.	Moisture (g/100g)	Protein (g/100g)	Fat (g/100g)	Fiber (g/100g)	Total ash (g/100g)	CHO (g/100g)	Energy (Kcal/100 g)
RwFB	6.7± 0.13	9.54±0.24 ^b	3.36±0.19 ^b	2.70±0.35 ^b	2.51±0.02 ^{bc}	75.25±0.63 ^a	369.69±2.05 ^{bc}
RaFB	6.39±0.17 ^c	9.28±0.15 ^b	3.87±0.55 ^a	2.90±0.41 ^a	2.54±0.04 ^{ab}	75.01±1.69 ^a	372.39±3.07 ^b
SFB	7.02±0.37 ^a	9.68±0.30 ^b	3.14±0.12 ^b	2.79±0.37 ^{ab}	2.46±0.04 ^c	74.92±2.11 ^b	366.95±7.05 ^c
GFB	6.21±0.05 ^d	10.07±0.41 ^a	3.81±0.21 ^a	2.96±0.13 ^a	2.57±0.03 ^a	75.52±0.65 ^a	376.95±0.55 ^a

Any two means in the same column not followed by the same letters are significantly different ($P<0.05$).

4.3.5 Total ash contents

The effect of blending ratio on the total ash content of the blended injera is shown in Table 4.4.1. It ranged from 2.48-2.57(g/100g) respectively with levels of 10% blends and control (Table 4.3.1). The total ash content of control injera gave significantly ($p<0.05$) higher mean than 5 and 10 % blended injera. As the amounts of fenugreek flour in the formulation increases, the amount of total ash in injera decrease. This is may be due to the highly reduction of ash content of processed fenugreek.

Total ash content of the treatments ranged from 2.46-2.57 (g/100g) respectively for soaked and germinated fenugreek supplemented injera (Table 4.3.2). The total ash content of germinated fenugreek supplemented injera gave significantly ($p<0.05$) higher mean followed by roasted fenugreek supplemented injera which also significantly differed from soaked fenugreek supplemented injera. However, ash content of roasted and raw fenugreek supplemented injera varied slightly significantly ($P>0.05$). Loss in ash might be due to leaching of soluble inorganic salts into the processing water during the soaking of the sample.

4.3.6 Carbohydrate content

The mean carbohydrate content of the blended injera ranged from 73.57-77.15(g/100g) respectively with levels of 10% blends and control (Table 4.3.1). The carbohydrate content of control injera gave significantly ($p<0.05$) higher mean than 5 and 10 % blended injera. As the amounts of fenugreek flour in the formulation increases, the amount of carbohydrate in injera decrease. This decreasing effect is attributed to the amount of the carbohydrate content of the fenugreek flour (41.27%) being highly lower than tef flour (69.66 %).

Carbohydrate content of the treatments ranged from 74.92-75.52(g/100g) respectively for soaked and germinated fenugreek supplemented injera (Table 4.3.2). The carbohydrate content of germinated, roasted and raw fenugreek supplemented injera gave significantly ($p<0.05$) higher mean than soaked fenugreek supplemented injera. However, carbohydrate content of germinated raw and roasted fenugreek supplemented injera varied slightly significantly ($P>0.05$). A slight decrease in the carbohydrate content was observed. The reason is probably due to a slight increase in the other nutrients of the injera having decreased the proportion of carbohydrate contents.

4.3.7 Total gross energy

The mean gross energy of the blended injera ranged from 368.78-375.26(g/100g) respectively with levels of 10% blends and control (Table 4.3.1). The gross energy of control injera gave significantly ($p<0.05$) higher mean followed by 5% blended injera which also significantly differed from 10% blended injera. As the amounts of fenugreek flour in the formulation increases, the amount of gross energy in injera decrease. This is may be due to the lower carbohydrate content of fenugreek flour.

Gross energy of the treatments ranged from 366.95-376.95(g/100g) respectively for soaked and germinated fenugreek supplemented injera (Table 4.3.2). The gross energy of germinated

fenugreek supplemented injera gave significantly ($p<0.05$) higher mean followed by roasted fenugreek supplemented injera which also significantly differed from soaked fenugreek supplemented injera. However, gross energy of raw fenugreek supplemented injera was no significantly differ ($P>0.05$) from roasted and soaked fenugreek supplemented injera. A slight change in the gross energy level was observed .The reason is probably due to a slight change in the other nutrients of the injera.

Table4.3. 3 Effect of processing*blending on proximate composition of supplemented injera

Blend proportion	Moisture	Protein	Fat	Crude fiber	Ash	Carbohydrate	Gross energy
Control(<i>Tef</i>)	6.32±0.05	9.09±0.22	3.26±0.21	1.61±0.07	2.57±0.03	77.15±0.42	375.26±0.88
T:RwF							
95:5	7.04±0.05	9.31±0.22	3.79±0.21	3.03±0.07	2.48±0.03	74.48±0.42	369.27±0.88
95:10	6.74±0.05	10.21±0.22	3.03±0.21	3.45±0.07	2.46±0.03	74.12±0.42	364.55±0.88
T:RaF							
95:5	6.88±0.05	9.11±0.22	3.97±0.21	3.38±0.07	2.46±0.03	74.20±0.42	368.91±0.88
90:10	5.96±0.05	9.66±0.22	4.40±0.21	3.70±0.07	2.59±0.03	73.69±0.42	372.98±0.88
T:SF							
95:5	6.55±0.05	9.38±0.22	3.10±0.21	3.42±0.07	2.42±0.03	75.13±0.42	365.95±0.88
90:10	8.19±0.05	10.58±0.22	3.05±0.21	3.33±0.07	2.39±0.03	72.47±0.42	359.65±0.88
T:GF							
95:5	6.08±0.05	9.96±0.22	4.05±0.21	1.94±0.07	2.64±0.03	75.37±0.42	377.63±0.88
90:10	6.22±0.05	11.21±0.22	4.11±0.21	2.32±0.07	2.49±0.03	74.02±0.42	377.94±0.88

Table 4.3.3.shows that the moisture, protein, fat and crude fibre contents increased with increasing fenugreek (raw, roasted, soaked and germinated) flour in *Tef* flour, whereas ash and total carbohydrate contents decreased from 2.57 % to 2.39 % and 77.15 % (control injera) to 72.47 % (soaked fenugreek- supplemented injera) respectively. Control injera had 9.09 % protein content. In the case of supplemented injera, it ranged from 9.31 % to 10.21 %, 9.11–9.66 %, 9.38-10.58 % and9.96–11.21 % in raw, roasted, soaked and germinated fenugreek based injera respectively. The increase in protein content of fenugreek supplemented injera might be the result of the appreciably higher protein content of fenugreek. Sharma and Chauhan (2000) also reported significantly ($P < 0.05$) higher protein content of breads prepared from blends of wheat-fenugreek flours.

4.4 minerals content of supplemented injera.

4.4.1 Calcium

In the present study, mean calcium content of the blended injera ranged from 173.43-266.66(g/100g) respectively with levels of control and 10% blends (Table 4.4.1). The calcium content of 10% blended injera gave significantly ($p<0.05$) higher mean followed by 5% blended injera which also significantly differed from control injera. As the amounts of fenugreek flour in the formulation increases, the amount of calcium in injera increase. This might be attributed to the direct replacement of tef flour by higher mineral contents of treated and untreated fenugreek flour.

Calcium content of the treatments ranged from 172.62-306.87(g/100g) respectively for germinated and soaked fenugreek supplemented injera (Table 4.4.2). The calcium content of soaked fenugreek supplemented injera gave significantly ($p<0.05$) higher mean followed by roasted fenugreek supplemented injera which also significantly differed from raw and germinated fenugreek supplemented injera. However, calcium content of raw and germinated fenugreek supplemented injera was no significantly differ ($P>0.05$).

4.4.2 Iron

The result of of blending ratio effects on iron content of fenugreek supplemented injera is shown in Tables 4.4.1. It ranged from 10.99-12.79(g/100g) respectively with levels of 5% and 10% blends (Table 4.4.1). The iron content of 10% blended injera gave significantly ($p<0.05$) higher mean followed by control injera which also significantly differed from 5% blended injera. As the amounts of fenugreek flour in the formulation increases, the amount of iron in injera increase. This might be attributed to the direct replacement of tef flour by higher mineral contents of treated and untreated fenugreek flour.

Table 4.4.1 Effect of blending ratio on minerals contents of supplemented injera

Blend proportion	Calcium	Iron	Zinc
100% <i>Tef</i>	173.43±0.23 ^c	11.75±0.19 ^b	2.72±0.02 ^a
5%FB	203.20±2.27 ^b	10.99±0.27 ^c	2.21±0.28 ^b
10% FB	266.66±4.01 ^a	12.79±0.29 ^a	1.99±0.43 ^c

Any two means in the same column not followed by the same letters are significantly different (P<0.05).

Iron content of the treatments ranged from 11.40-12.41(g/100g) respectively for germinated and roasted fenugreek supplemented injera (Table 4.4.2). The iron content of roasted and raw fenugreek supplemented injera gave significantly (p<0.05) higher mean followed by soaked fenugreek supplemented injera which also significantly differed from germinated fenugreek supplemented injera. However, calcium content of raw and roasted fenugreek supplemented injera was no significantly differ (P>0.05).

Table 4.4.2 Effect of processing on minerals contents of supplemented injera

Treatments	Calcium	Iron	Zinc
RwFB	173.29±1.06 ^c	12.13±0.44 ^a	3.14±0.16 ^a
RaFB	204.90±1.44 ^b	12.41±0.40 ^a	2.00±0.25 ^c
SFB	306.87±5.01 ^a	11.44±0.25 ^b	1.50±0.40 ^d
GFB	172.67±3.22 ^c	11.40±0.48 ^c	2.59±0.07 ^b

Any two means in the same column not followed by the same letters are significantly different (P<0.05).

4.4.3 Zinc

The mean zinc content of the blended injera ranged from 1.99-2.72(g/100g) respectively with levels of 10% blends and control (Table 4.4.1). The zinc content of control injera injera gave significantly (p<0.05) higher mean followed by 5% blended injera which also significantly differed from 10 %

blended injera. As the amounts of fenugreek flour in the formulation increases, the amount of zinc in injera decrease.

Zinc content of the treatments ranged from 1.50-3.14(g/100g) respectively for soaked and raw fenugreek supplemented injera (Table 4.4.2). The zinc content of raw fenugreek supplemented injera gave significantly ($p<0.05$) higher mean followed by germinated fenugreek supplemented injera which also significantly differed from roasted and soaked fenugreek supplemented injera. Loss in zinc content might be due to leaching of zinc into the processing water during the soaking of the sample.

Table 4.4.3. Effect of processing*blending on mineral content of supplemented injera

Blend proportion	Calcium	Iron	Zinc
Control(<i>Tef</i>)	173.43±0.94	11.75±0.33	2.72±0.10
T:RwF			
95:5	144.27±0.94	11.24±0.33	3.13±0.10
95:10	202.17±0.94	13.39±0.33	3.57±0.10
T:RaF			
95:5	205.17±0.94	11.90±0.33	1.91±0.10
90:10	236.09±0.94	13.57±0.33	1.37±0.10
T:SF			
95:5	262.74±0.94	10.81±0.33	1.19±0.10
90:10	299.84±0.94	11.76±0.33	0.59±0.10
T:GF			
95:5	163.53±0.94	10.00±0.33	2.59±0.10
90:10	181.05±0.94	12.44±0.33	2.45±0.10

Total minerals in control as well as supplemented injera are presented in Table 4.4.3. Control injera had low total Ca (173.43 mg/100 g), Zn (2.72 mg/ 100 g) and Fe (11.7 mg/100 g) contents. Mineral contents increased on increasing the substitution of raw and processed fenugreek flour in tef flour. Raw fenugreek supplemented injera manifested higher contents of total Ca, Fe and Zn, followed by roasted, soaked and germinated fenugreek-supplemented injera. However the content of total Zn decreased on increasing the substitution of processed fenugreek flour in tef flour.

4.5 Anti-nutritional factors

The anti-nutritional factors (phytate and tannin) contents of the control and supplemented injera are shown in table 4.4.1, 4.4.2 and 4.4.3

4.5.1 Tannin

In the present study, mean tannin content of the blended injera ranged from 2.05-11.7(g/100g) respectively with levels of control and 10% blends (Table 4.5.1). The tannin content of 10% blended injera gave significantly ($p<0.05$) higher mean followed by 5% blended injera which also significantly differed from control injera. As the amounts of fenugreek flour in the formulation increases, the amount of tannin in injera increase. The increased tannin content of the injera seems to be the direct effect of replacement of tef flour with fenugreek flour, which has of high content of tannin.

Table 4.5.1 Effect of blending ratio on ant nutritional factors of supplemented injera

Blend Proportion	tannin	phytate
100% <i>Tef</i>	2.05±0.01 ^c	150.62±0.73 ^a
5%FB	9.37±1.46 ^b	133.83±5.75 ^b
10% FB	11.7±1.46 ^a	131.28±6.49 ^c

Any two means in the same column not followed by the same letters are significantly different ($P<0.05$).

Tannin content of the treatments ranged from 4.31-11.25(g/100g) respectively for germinated and raw fenugreek supplemented injera (Table 4.5.2). The tannin content of raw fenugreek supplemented injera gave significantly ($p<0.05$) higher mean followed by roasted fenugreek supplemented injera which also significantly differed from soaked and germinated fenugreek supplemented injera. The results revealed that there was a significant reduction in the tcontent of tannin during roasting, soaking and germinating treatments. This is in agreement with the result reported by shalini and sudes (2002).

4.5.2 Phytate

The mean phytate content of the blended injera ranged from 131.28-150.62(g/100g) respectively with levels of 10% blends and control (Table 4.5.1). The phytate content of control injera gave significantly ($p<0.05$) higher mean followed by 5% blended injera which also significantly differed from 10% blended injera. The amount of phytate in injera decreased progressively with increasing level of fenugreek flour. This might be due to the higher reduction of phytic acid by different processing methods.

Table 4.5.2 Effect of processing on ant nutritional factors of supplemented injera

Treatments	tannin	phytate
RwFB	11.25±2.94 ^a	153.46±1.23 ^a
RaFB	8.67±2.12 ^b	128.40±7.07 ^c
SFB	6.64±1.62 ^c	144.13±2.47 ^b
GFB	4.31±0.77 ^d	128.32±7.10 ^c

Any two means in the same column not followed by the same letters are significantly different ($P<0.05$).

Phytate content of the treatments ranged from 128.32-153.46(g/100g) respectively for germinated and raw fenugreek supplemented injera (Table 4.5.2). The phytate content of raw fenugreek supplemented injera gave significantly ($p<0.05$) higher mean followed by soaked fenugreek supplemented injera which also significantly differed from roasted and germinated fenugreek supplemented injera. Reduction in the phytic acid content during soaking, roasting or germination has been reported by Chau and Cheung (1997).

Table4.5. 3 Effect of processing*blending on Ant nutritional factors of supplemented injera

Blend proportion	Tannin	Phytate
Control(<i>Tef</i>)	2.05±0.15	150.62±1.22
T:RwF		
95:5	14.76±0.15	153.12±1.22
95:10	16.95±0.15	156.63±1.22
T:RaF		
95:5	11.05±0.15	118.93±1.22
90:10	12.90±0.15	115.66±1.22
T:SF		
95:5	7.00±0.15	144.29±1.22
90:10	10.88±0.15	137.48±1.22
T:GF		
95:5	4.66±0.15	118.97±1.22
90:10	6.23±0.15	115.38±1.22

The control (*Tef*) injera had 2.05 mg/100 g tannin and this increased with rise in the levels of fenugreek flours (Table 4.5.3). The maximum increase of tannin over the control was found in injera containing raw fenugreek flour at 10% level (16.95 mg/100 g) while minimum increase was found in germinated fenugreek flour (6.23 mg/100 g), over the control. The increased tannin content of the injera seems to be the direct effect of replacement of Tef flour with fenugreek flour, which has of high content of tannin (Hooda & Jood, 2003). Contrary to this, phytic acid contents decreased progressively with increasing level of processed fenugreek flour, however slightly increase with supplementation of raw fenugreek. Among the supplemented injera, soaked fenugreek flour-supplemented injera had contained lower contents of phytic acid at 5% and 10% levels. This might be due to the higher reduction of phytic acid by different processing methods.

4.6 Molar ratios of Ca: Phy, Phy: Fe, phy:Zn and [Ca] [Phy]/[Zn]

The molar ratios for calcium, zinc, Iron and phytate were calculated to evaluate the effects of the higher levels of phytate in the bioavailability of dietary minerals. Bioavailability is the ability of the body to digest and absorb the mineral in the food consumed. Phytic acid exerts its inhibitory effect

on the absorption of zinc and iron by forming insoluble complexes in the gut under physiological condition. The formation of such chelates depends on the ratio of the content of zinc, iron or calcium relative to that of phytate in the food (Umeta et al., 2005). The calculated values are also compared with the reported critical toxicity values for these ratios. The calculated Ca: Phy, Ox: Ca,Phy: Zn, Phy: Fe and $[Ca] [Phy]/ [Zn]$ molar ratios of control and supplemented injera is shown in Table 4.6.1 and 4.6.2.

4.6.1 [Calcium]/ [Phytate] molar ratios

The molar ratios of Ca:Phy in control, 5 % and 10 % supplemented injera were19,24.19and29.22 respectively. The mean Ca:Phy molar ratio of fenugreek supplemented injera was significantly ($P<0.05$) higher than the control injera. Phytic acids markedly decrease Ca bioavailability and the Ca:Phy molar ratio has been proposed as an indicator of Ca bioavailability. The Ca: Phy molar ratios >6 , indicative of poor calcium bioavailability (Oladimeji *et al.*, 2000). The values in the present study were higher than the reported critical molar ratio of Ca:Phy, indicating that absorption of calcium adversely affected by phytate in these supplementation.

Table 4.6.1 Effect of blending ratio on calcium: phytate, phytate:iron, phytate:zinc and ([calcium][phytate]):zinc molar ratio of supplemented injera

Treatments	Calcium: phytate	Phytate:Iron	Phytate:Zinc	([calcium][phytate]):zinc
(100%) <i>Tef</i>	19.00±0.12 ^c	1.09±0.02 ^a	5.45±0.01 ^c	0.24±0.00 ^c
5% FB	24.19±2.15 ^b	1.04±0.05 ^a	6.87±1.14 ^b	0.36±0.09 ^b
10% FB	29.22±2.23 ^a	0.87±0.05 ^b	10.18±2.89 ^a	0.66±0.24 ^a

Any two means in the same column not followed by the same letters are significantly different ($P<0.05$).

With in different treatments, the mean Ca:Phy molar ratio of fenugreek supplemented injera was significantly ($P<0.05$) higher than the raw supplemented injera. The Ca: Phy molar ratios in the

present study were higher than the reported critical molar ratio of Ca:Phy, this is indicating that absorption of calcium adversely affected by phytate in these treated and untreated fenugreek supplementation.

4.6.2 [Phytate]/[Iron] molar ratios

The molar ratios of Phy:Fe in control, 5 % and 10 % supplemented injera were 1.09, 1.04 and 0.87 respectively. The mean Phy:Fe molar ratio of 10% fenugreek supplemented injera was significantly ($P < 0.05$) higher than the control injera.

With in different treatments, the mean Phy:Fe molar ratio of raw fenugreek supplemented injera was significantly ($P < 0.05$) higher than the roasted and germinated fenugreek supplemented injera.

However, the mean Phy:Fe molar ratio of raw and soaked flour samples varied slightly significantly ($P > 0.05$). The phytate: iron molar ratio is greater than 0.15, regarded as indicative of poor iron bioavailability (Umata et al., 2005). This result indicated that, the phytate: iron molar ratios in different blending ratio and processing methods were greater than the critical value, which implies the absorption of iron from treated and untreated fenugreek supplemented injera inhibited by phytate and as a result the bioavailability of iron is poor.

4.6.3 [Phytate]/ [Zinc] molar ratios

The molar ratios of Phy:Zn in control, 5 % and 10 % supplemented injera were 5.45, 6.87 and 10.18 respectively. The mean Phy:Zn molar ratio of fenugreek supplemented injera was significantly ($P < 0.05$) higher than the control injera.

Table 4.6.2 Effect of processing on calcium: phytate, phytate:iron, phytate:zinc and ([calcium][phytate]):zinc contents of supplemented injera

Treatments	Calcium: phytate	Phytate:Iron	Phytate:Zinc	([calcium][phytate]):zinc
RwFB	18.62±18.62 ^d	1.08±0.03 ^a	4.96±0.19 ^c	0.21±0.01 ^c
RaFB	27.05±2.72 ^b	0.89±0.07 ^c	6.68±0.62 ^b	0.35±0.05 ^b
SFB	28.35±3.15 ^a	1.07±0.03 ^a	13.49±3.26 ^a	0.92±0.28 ^a
GFB	22.53±1.26 ^c	0.96±0.06 ^b	4.88±0.21 ^c	0.21±0.01 ^c

Any two means in the same column not followed by the same letters are significantly different (P<0.05).

With in different treatments, the mean Phy:Zn molar ratio of roasted and soaked fenugreek supplemented injera was significantly (P<0.05) lower than the raw fenugreek supplemented injera. However, the mean Phy:Zn molar ratio of raw and germinated fenugreek supplemented injera varied slightly significantly (P>0.05). Phytate: zinc molar ratios >15, indicative of poor zinc bioavailability (Morris and Ellis, 1989). The values of control, different blending ratio and with in different processing methods were lower than the critical molar ratios of Phy:Zn, which indicates the availability of zinc is good in 5 and 10% blending proportion and different processing methods.

4.6.4 [Calcium][Phytate]/ [Zinc] molar ratios

The ratio of [Ca] x [Phytate]: [Zn] is a better predictor of Zn availability and if the value were greater than half mol/kg, there would be interferences with the availability of Zn. (Umeta et al., 2005). In this result, [Ca] x [Phytate]: [Zn] values were greater than 0.50 mol/kg in 10% fenugreek supplemented injera and soaked fenugreek supplemented injera while other [Ca] x [Phytate]: [Zn] values were lower than to 0.50 mol/kg. Which indicates the availability of zinc is good at 5% blending proportion and due to processed fenugreek supplemented injera except soaking.

Table 4.6.3 Effect of processing*blending on calcium: phytate, phytate:iron, phytate:zinc and ([calcium][phytate]):zinc contents of supplemented injera

Blend proportion	caphy	phyiron	phyzinc	caphyovzn
Control(<i>Tef</i>)	19.00±0.24	1.09±0.04	5.45±0.44	0.24±0.03
T:RwF				
95:5	15.55±0.24	1.16±0.04	4.84±0.44	0.18±0.03
95:10	21.30±0.24	0.99±0.04	4.57±0.44	0.22±0.03
T:RaF				
95:5	28.47±0.24	0.85±0.04	6.14±0.44	0.31±0.03
90:10	33.68±0.24	0.72±0.04	8.44±0.44	0.50±0.03
T:SF				
95:5	30.05±0.24	1.13±0.04	11.95±0.44	0.79±0.03
90:10	35.99±0.24	0.99±0.04	23.07±0.44	1.73±0.03
T:GF				
95:5	22.68±0.24	1.01±0.04	4.54±0.44	0.19±0.03
90:10	25.90±0.24	0.79±0.04	4.66±0.44	0.21±0.03

The molar ratios of control, different blending ratio and with in different processing methods were lower than the critical molar ratios of each mineral, except the molar ratio of Zn. Which indicates the availability of zinc is good, but the availability of Calcium and Iron was poor.

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study attempted to investigate the possibility of using fenugreek flour for the production of injera by blending with tef flour. The effect of blend proportion of fenugreek and tef flour and some processing methods on the physicochemical properties and sensory qualities of fenugreek supplemented injera was studied.

The results of this study showed that fenugreek flour could be blended with tef flour to produce injera with required physicochemical properties and acceptable sensory qualities. It was found that the more the fenugreek flour in the composite, the more the protein, fat, fiber and ash content of the injera. Incorporation of the soaked and germinated fenugreek flour up to 10% resulted in injera of comparable in most quality parameters considered (nutritional and sensory parameters) with those produced using 100% tef flour. The study also indicated that soaking and germinating methods resulted in better physical characteristics and sensory qualities of injera as compared to roasting. Moreover, the study revealed that blending tef flour with treated (roasted, soaked and germinated) fenugreek flour in the injera formulation could significantly reduce the ant nutritional factors (phytate and tannin contents).

It may be also inferred from the present study that nutritional quality of fenugreek seeds can be improved through processing methods, and bitterness can also be reduced to some extent. Therefore, the use of processed fenugreek flour can be exploited in functional and nutritional foods as well as a therapeutic agent in various bakery products.

From the results of this study, fenugreek-tef flour injera are both nutritious and exhibited acceptable sensory quality. Thus it could be concluded that supplementation of fenugreek seed flour on the tef flour enhances the nutritive value of products. Hence, development and

consumption of such therapeutic bakery products would help to raise the nutritional status of the population.

5.2 Recommendations

The following recommendations are made based on the study conducted and findings on fenugreek supplemented injera.

- Injera which is produced from fenugreek-tef composite flour should get special attention and emphasis by the consumers due to its nutritional value. Women, who produce injera should be encouraged to utilize the potential of fenugreek flour there by diversifying their products for better service of the consumers.
- Supplementation of fenugreek on tef flour for injera production should also be encouraged since it has a lot of medicinal value which makes it a very good functional food.
- Food processing industries should use fenugreek in order to enrich their products with protein, fat, fibre, minerals and other nutrients.
- Agricultural institutes should promote and propagates the nutritional and medicinal value of fenugreek there by conducting further studies, and providing selected seeds for the farmers.
- Health institutions, should advise the people to include this legume in their foods and enjoy its medicinal value.
- It is also recommended for other researchers to conduct a research on processing methods to reduce the bitterness and odor of this legume other than roasting, soaking and germination, which are suggested in this study.

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Appendix

1. Appendix on Proximate composition of the supplemented injera.

1.1. Tests of Between-Subjects Effects on moisture contents of the supplemented injera

Dependent Variable: Moisture

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.852 ^a	11	.714	139.056	.000
Intercept	1038.598	1	1038.598	2.023E5	.000
Processing	2.295	3	.765	149.046	.000
Blending	.865	2	.432	84.227	.000
processing * blending	4.692	6	.782	152.336	.000
Error	.062	12	.005		
Total	1046.511	24			
Corrected Total	7.914	23			

a. R Squared = .992 (Adjusted R Squared = .985)

1.2. Tests of Between-Subjects Effects on protein contents of the supplemented injera

Dependent Variable: protein

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10.881 ^b	11	.989	10.633	.000
Intercept	2231.832	1	2231.832	2.399E4	.000
processing	1.937	3	.646	6.939	.006
blending	7.648	2	3.824	41.103	.000
processing * blending	1.297	6	.216	2.324	.101
Error	1.116	12	.093		
Total	2243.829	24			
Corrected Total	11.998	23			

b. R Squared = .907 (Adjusted R Squared = .822)

1.3. Tests of Between-Subjects Effects on fat contents of the supplemented injera

Dependent Variable: Fat

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.180 ^c	11	.471	5.288	.004
Intercept	301.380	1	301.380	3.384E3	.000
processing	2.280	3	.760	8.534	.003
blending	1.015	2	.507	5.697	.018
processing * blending	1.885	6	.314	3.528	.030
Error	1.069	12	.089		
Total	307.629	24			
Corrected Total	6.249	23			

c. R Squared = .829 (Adjusted R Squared = .672)

1.4. Tests of Between-Subjects Effects on fibre contents of the supplemented injera

Dependent Variable: fibre

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.708 ^d	11	1.519	176.871	.000
Intercept	160.218	1	160.218	1.866E4	.000
processing	3.268	3	1.089	126.859	.000
blending	11.641	2	5.820	677.783	.000
processing * blending	1.799	6	.300	34.907	.000
Error	.103	12	.009		
Total	177.029	24			
Corrected Total	16.811	23			

d. R Squared = .994 (Adjusted R Squared = .988)

1.5. Tests of Between-Subjects Effects on ash contents of the supplemented injera

Dependent Variable: ash

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.133 ^e	11	.012	6.109	.002
Intercept	152.292	1	152.292	7.687E4	.000
processing	.040	3	.013	6.680	.007
blending	.035	2	.018	8.903	.004
processing * blending	.058	6	.010	4.892	.009
Error	.024	12	.002		
Total	152.449	24			
Corrected Total	.157	23			

e. R Squared = .848 (Adjusted R Squared = .710)

1.6. Tests of Between-Subjects Effects on carbohydrate contents of the supplemented injera

Dependent Variable: carbohydrate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	58.304 ^f	11	5.300	15.256	.000
Intercept	135626.360	1	135626.360	3.904E5	
processing	1.296	3	.432	1.244	.337
blending	53.026	2	26.513	76.311	.000
processing * blending	3.982	6	.664	1.910	.160
Error	3.982	6	.664	1.910	.160
Total	135688.833	24			
Corrected Total	62.473	23			

e. R Squared = .848 (Adjusted R Squared = .710)

1.7. Tests of Between-Subjects Effects on gross energy of the supplemented injera

Dependent Variable: Gross energy

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	738.609 ^g	11	67.146	43.269	.000
Intercept	3312192.433	1	3312192.433	2.134E6	
processing	326.345	3	108.782	70.098	
blending	181.445	2	90.722	58.461	
processing * blending	230.819	6	38.470	24.790	.000
Error	18.622	12	1.552		
Total	3312949.664	24			
Corrected Total	757.231	23			

g. R Squared = .975 (Adjusted R Squared = .953)

2. Appendix on sensory characteristics of the supplemented injera.

2.1. Tests of Between-Subjects Effects on appearance of the supplemented injera

Dependent Variable: Appearance

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1456.900 ^a	19	76.679	90.447	.000
Intercept	5100.500	1	5100.500	6.016E3	.000
processing	181.380	3	60.460	71.316	.000
blending	1147.250	4	286.813	338.311	.000
processing * blending	128.270	12	10.689	12.608	.000
Error	152.600	180	.848		
Total	6710.000	200			
Corrected Total	1609.500	199			

a. R Squared = .905 (Adjusted R Squared = .895)

2.2. Tests of Between-Subjects Effects on color of the supplemented injera

Dependent Variable: Color

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1657.255 ^b	19	87.224	111.429	.000
Intercept	5130.845	1	5130.845	6.555E3	.000
processing	205.375	3	68.458	87.456	.000
blending	1300.430	4	325.108	415.325	.000
processing * blending	151.450	12	12.621	16.123	.000
Error	140.900	180	.783		
Total	6929.000	200			
Corrected Total	1798.155	199			

b. R Squared = .922 (Adjusted R Squared = .913)

2.3. Tests of Between-Subjects Effects on taste of the supplemented injera

Dependent Variable: Taste

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1511.480 ^c	19	79.552	63.192	.000
Intercept	4305.920	1	4305.920	3.420E3	.000
processing	112.680	3	37.560	29.836	.000
blending	1329.130	4	332.283	263.949	.000
processing * blending	1329.130	4	332.283	263.949	
Error	226.600	180	1.259		
Total	6044.000	200			
Corrected Total	1738.080	199			

c. R Squared = .870 (Adjusted R Squared = .856)

2.4. Tests of Between-Subjects Effects on odor of the supplemented injera

Dependent Variable: Odor

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1649.175 ^d	19	86.799	74.862	.000
Intercept	4753.125	1	4753.125	4.099E3	.000
processing	156.015	3	52.005	44.853	.000
blending	1404.250	4	351.063	302.785	.000
processing * blending	88.910	12	7.409	6.390	.000
Error	208.700	180	1.159		
Total	6611.000	200			
Corrected Total	1857.875	199			

d. R Squared = .888 (Adjusted R Squared = .876)

2.5. Tests of Between-Subjects Effects on texture of the supplemented injera

Dependent Variable: Texture

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1484.600 ^e	19	78.137	61.850	.000
Intercept	5000.000	1	5000.000	3.958E3	.000
processing	163.000	3	54.333	43.008	.000
blending	1205.350	4	301.338	238.526	.000
processing * blending	116.250	12	9.688	7.668	.000
Error	227.400	180	1.263		
Total	227.400	180	1.263		
Corrected Total	227.400	180	1.263		

e. R Squared = .867 (Adjusted R Squared = .853)

2.6. Tests of Between-Subjects Effects on overall acceptability of the supplemented injera

Dependent Variable: Overall acceptability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1439.080 ^f	19	75.741	64.736	.000
Intercept	4920.320	1	4920.320	4.205E3	.000
processing	117.720	3	39.240	33.538	.000
blending	1252.930	4	313.233	267.720	.000
processing * blending	68.430	12	5.703	4.874	.000
Error	210.600	180	1.170		
Total	6570.000	200			
Corrected Total	1649.680	199			

f. R Squared = .872 (Adjusted R Squared = .859)

3. Appendix on Metal contents of the supplemented injera.

3.1. Tests of Between-Subjects Effects on calcium contents of the supplemented injera

Dependent Variable: Calcium

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45691.220 ^a	11	4153.747	2.333E3	.000
Intercept	950881.755	1	950881.755	5.341E5	.000
processing	21215.800	3	7071.933	3.972E3	.000
blending	13019.449	2	6509.724	3.656E3	.000
processing * blending	11455.971	6	1909.328	1.072E3	.000
Error	21.366	12	1.780		
Total	996594.341	24			
Corrected Total	45712.586	23			

a. R Squared = 1.000 (Adjusted R Squared = .999)

3.2. Tests of Between-Subjects Effects on Iron contents of the supplemented injera

Dependent Variable: Iron

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21.208 ^b	11	1.928	8.718	.000
Intercept	3365.875	1	3365.875	1.522E4	.000
processing	4.559	3	1.520	6.871	.006
blending	13.099	2	6.549	29.615	.000
processing * blending	3.551	6	.592	2.676	.069
Error	2.654	12	.221		
Total	3389.738	24			
Corrected Total	23.862	23			

b. R Squared = .889 (Adjusted R Squared = .787)

3.3. Tests of Between-Subjects Effects on Zinc contents of the supplemented injera

Dependent Variable: Zinc

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.548 ^c	11	1.504	69.366	.000
Intercept	127.651	1	127.651	5.886E3	.000
processing	9.089	3	3.030	139.696	.000
blending	2.233	2	1.116	51.476	.000
processing * blending	5.226	6	.871	40.164	.000
Error	.260	12	.022		
Total	144.459	24			
Corrected Total	16.808	23			

c. R Squared = .985 (Adjusted R Squared = .970)

4. Appendix on Ant nutritional factors of the supplemented injera.

4.1. Tests of Between-Subjects Effects on tannin content of the supplemented injera

Dependent Variable: Tannin

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	646.681 ^a	11	58.789	1.378E3	.000
Intercept	1429.744	1	1429.744	3.350E4	.000
processing	156.920	3	52.307	1.226E3	.000
blending	408.075	2	204.038	4.781E3	.000
processing * blending	81.686	6	13.614	319.023	.000
Error	.512	12	.043		
Total	2076.938	24			
Corrected Total	647.194	23			

a. R Squared = .999 (Adjusted R Squared = .998)

4.2. Tests of Between-Subjects Effects on phytate content of the supplemented injera

Dependent Variable: Phytate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5970.722 ^b	11	542.793	182.433	.000
Intercept	460886.593	1	460886.593	1.549E5	.000
processing	2765.398	3	921.799	309.818	.000
blending	1766.214	2	883.107	296.813	.000
processing * blending	1439.109	6	239.852	80.614	.000
Error	35.704	12	2.975		
Total	466893.018	24			
Corrected Total	6006.425	23			

b. R Squared = .994 (Adjusted R Squared = .989)

5. Appendix on Phytate-metal molar ratio of the supplemented injera.

5.1. Tests of Between-Subjects Effects on Cal-phy molar ratio of the supplemented injera

Dependent Variable: calcium-phytate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	955.502 ^a	11	86.864	760.483	.000
Intercept	13980.262	1	13980.262	1.224E5	.000
processing	355.692	3	118.564	1.038E3	.000
blending	417.281	2	208.640	1.827E3	.000
processing * blending	182.529	6	30.422	266.337	.000
Error	1.371	12	.114		
Total	14937.135	24			
Corrected Total	14937.135	24			

a. R Squared = .999 (Adjusted R Squared = .997)

5.2. Tests of Between-Subjects Effects on phytate-Iron mole ratio of supplemented injera.

Dependent Variable: phytate-Iron

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.438 ^b	11	.040	14.507	.000
Intercept	24.006	1	24.006	8.738E3	.000
processing	.153	3	.051	18.611	.000
blending	.203	2	.101	36.893	.000
processing * blending	.082	6	.014	4.993	.009
Error	.033	12	.003		
Total	24.478	24			
Corrected Total	24.478	24			

b. R Squared = .930 (Adjusted R Squared = .866)

5.3. Tests of Between-Subjects Effects on phytate-Zinc mole ratio of supplemented injera.

Dependent Variable: phytate-Zinc

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	628.169 ^c	11	57.106	148.944	.000
Intercept	1350.482	1	1350.482	3.522E3	.000
processing	299.216	3	99.739	260.138	.000
blending	94.302	2	47.151	122.978	.000
processing * blending	234.652	6	39.109	102.003	.000
Error	4.601	12	.383		
Total	1983.252	24			
Corrected Total	632.770	23			

c. R Squared = .993 (Adjusted R Squared = .986)

5.4. Tests of Between-Subjects Effects on cal-phytate/Zinc mole ratio of supplemented injera.

Dependent Variable: Cal-phytate/Zinc

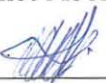
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.399 ^d	11	.400	216.798	.000
Intercept	4.269	1	4.269	2.315E3	.000
processing	4.269	1	4.269	2.315E3	.000
blending	4.269	1	4.269	2.315E3	.000
processing * blending	.772	2	.386	209.307	.000
Error	1.589	6	.265	143.545	.000
Total	8.690	24			
Corrected Total	4.421	23			

c. R Squared = .993 (Adjusted R Squared = .986)

DECLARATION

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

Name: Woinshet Abera Egigayhu

Signature: 

Date: 25/09/2003

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

Name: Dr. Gulelat Desse

Signature: 

Date: _____

Dr. Melese Abdisa

Signature: _____

Date: _____