ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

The Effect of processing on Nutritive values and Anti nutritional factors of two New Varieties of Tef *[Eragrotisis Tef (Zucc.)trotter] in Ethiopia*

By:- Demissie Teshome

A Thesis presented to the school of Graduate studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in food science and nutrition

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TABLE OF CONTENTS **Page**

List of Tables **Page**

List of Figures **Pages**

ACRONYMS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AAS	Atomic Absorption Spectrophotometer
CSA	Central Statics Agency
DZRAC	Debre Zeit Research and Agricultural Center
EHNRI	Ethiopia Health and Nutrition Research Institute
SD	Standard Deviation
SPSS	Statical Product and Service Solutions
FWT	Flour of WhiteTef
WTFB-72	White Tef Fermented for 72hr and Baked
WTF-72	White Tef Fermented for 72 hr
FRT	Flour of Red Tef
RTFB-72-	Red Tef Fermented for 72hr and Baked
RTF-72-	Red Tef Fermented for 72 hr

Abstract

The aim of this study was to determine the effect of processing on the nutritional values and anti nutritional factors of the two new varieties of Tef. White Tef (DZ-Cr-387, Qunicho) and Red Tef (DZ-01-99) in Ethiopia. Nutrition data of products were analyzed by proximate analysis. Moisture content, total ash, crude protein, crude fat, crude fiber contents of samples were determined and utilizable carbohydrates and energy were calculated from the obtained data of proximate analysis. It was found that 100gram of white tef consisted of 8.47 % moisture, 2.39% ash, 6.7% protein, and 3.1% fat, 5.3 % fiber, and 12.82mg/100g iron, 3.68mg/100g, 33.78mg/100g calcium, 51.27mg/100g of phosphorus. Similarly, 100g of red tef consisted of 8.4 % moisture, 2.45 % total ash, 6.36% protein, 3.63 % fat, 4.94% fiber and 12.08 mg/100g iron, 4.33mg/100g zinc, 36.13mg/100g calcium and 51.18% phosphorus. Significantly higher contents of protein, fat, ash, iron, calcium, phosphorus, phytate and tannins were found ($P<0.05$) for this two varieties of tef. The value for the phytate content of flour, fermented and baked white tef were 154. 50mg/100g, 142.15mg/100g and 115.27mg/100g and the values for the phytate content of flour fermented and baked red tef were 162.97mg/100g, 153.14mg/100g and 146.68mg/100g,respectively. Similarly, the tannin for the flour, fermented and baked white tef were 86.82 mg/100g, 80.42mg/ 100g and 78.40mg/100g and the tannin values of the flour, fermented and baked red tef were 80.23mg/100g, 74.43mg/100g and 76.51mg/100g, respectively. Thus the results indicated that both treatments on these two varieties decrease anti nutritional factors. Then one can conclude that fermentation and baking were most effective processing methods in reducing phytate and tannin contents.

Key words: Baking, Fermentation, Phytate and Tannin

1. Introduction

1.1 Back ground

For a sustainable and stable food production, maintaining genetic diversity and increasing nutritional content within and between crop types is increasingly being realized as the most logical and indispensable action. This is further emphasized by the unpredictable human food needs, tastes, technological demands, and various biotic and abiotic production constraints that are ever changing with environment and faced during crop production (Seifu, 1983).

In addition, the increasing demand for food, resulting from the rising number of world population is pushing agricultural production to marginal and sub marginal lands. These lands are characterized by limited moisture as well as unfavorable physical and chemical edaphic condition (Seifu, 1983).

Thus, apart from maintaining the diversity within and between crop types that do well under optimum production conditions, it is being realized that identifying, maintaining and utilizing hardy crop types that can grow under various stress conditions is essential. It is in this context that Tef (*Eragrostis tef),* a traditional crop that grows very well under various stress conditions and extensively used in Ethiopia (Taddesse, 1975; Seifu, 1983). But little is known elsewhere, deserves research attention and is saved as a human heritage. Hence, its cultivation should continue in Ethiopia, its potential be assessed for utilization in other

parts of the world, and its genetic resources, nutritional conditions and values be saved and passed over to future generations.

It is important to note that the major genetic diversities of tef existing at present are found only in Ethiopia (Taddesse, 1975; Seifu, 1983). For this reason, concerned international and local institutions should pay particular attention to this fact and do their best to collect and conserve tef germplasm and nutritional content so as to avoid an irreplaceable loss as well as to contribute towards the promotion of tef research to the benefit of mankind. After all, maintaining biodiversity, nutrient value is to the best interest of humanity. The purpose of this research paper thus is to document the status of a new variety of tef, white tef (DZ-Cr-387, Qunicho) and red tef (DZ-01-99) in terms of its nutritional and antinutritional factors.

1.2. Objective of the Study

1.2.1 General Objective

To analyze the effect of home based processing on nutritional values and antinutritonal factors of two new tef varieties.

1.2.2. Specific Objectives

- To determine the effect of fermentation and baking on antinutritional factors of new varieties of tef.
- To determine the effect of fermentation and baking on proximate composition and minerals of two new varieties of tef.
- To determine nutritional values of the two new varieties of tef.

2. Literature review

2.1. Over view of tef

Tef *[Eragrostis tef* (Zucc.) trotter] is one of the most important cereal crops in Ethiopia and accounts for about two-thirds of the daily protein intake in the diet of the population (Ethiopian Nutrition Survey 1959; Alemayehu Abebe, 1990). The area under tef cultivation is over one million hectares of land each year. Tef occupies 28% of the cultivated land under cereals, while sorghum occupies 20%, barley 17%, maize 16%, wheat 13%, millet 5%, and oats 1%. This clearly indicates the importance of tef in Ethiopia (CSO 1984; CSA, 2000).

Outside Ethiopia, there is a growing interest to utilize the crop. For example, small scale commercial production of the crop has already been started in few areas of the wheat belts of the United States and Australia. Tef had also been cultivated in South Africa as a forage crop for some time. Tef is one of the smallest grain in the world measuring only about 1/32 of an inch in diameter, approximately 150 grains equals the size of the kernel of wheat (Kuma and Mekonen, 1995).

2.1.1 Taxonomy and Morphology of tef

Tef belongs to Kingdom *planta* Division *Magnoliophyta* Class *Magnoliopsida* Order *Poales* Family poaceae, sub-family *Eragrostide,* tribe *Eragrosteae* and genus *Eragrostis.* The genus contains about 350 species in tropical and sub tropical regions (Constanza, 1974).

The crop has been given various scientific names at different times (Shaw 1976; Bekele, 1978). *Eragrostis tef (*Zucc.) Trotter in 1918, which is currently in use; *E. pilosa* (L.) p. beauv.var.tef (Zucc) in 1923; *E. pilosa* (L) p. beauv. Subsp. *abyssinica* (Jacq.) Aschers and Graben in 1900; *E. abyssinica* (jacq.) link in 1827; Cynodon abyssinicus (jacq.) rasp. In 1825; *Poa cerealis* Salisb in 1796; P.abyssinica Jacqin in 1781; and P. *Tef* Z uccagni in 1775.

Tef is a C_4, self- pollinated, chasmogamous annual cereal. It has a fibrous root system with mostly erect stems, although some cultivars are bending or elbowing types. The sheaths of tef are smooth, glabrous, open and distinctly shorter than the internodes. Its ligule's is very short and ciliated while its lamina is slender, narrow and nearly linear with elongated acute tips. It has an inflorescence panicle type showing different forms- from loose to compact, the latter appearing like a spike. Its spike lets have 2-12 florets. Each floret has a lemma, palea, three stamens, an ovary, and mostly two, in exceptional cases three, feathery stigmas. The cariopsis is 0.9-1.7 mm in length, and 0.7-1.0 mm in diameter, which is very small, and a color that varies from white to dark brown (Taddesse ,1975).

2.1.2 Center of origin and Diversity of tef

Several endemic and non- endemic species of *Eragrostis* exist, some of which are considered the wild relatives of tef, are found in Ethiopia. The genetic diversity for tef exists nowhere in the world except in Ethiopia, which indicates that tef originated and was

domesticated in Ethiopia it was identified that Ethiopia as the center of origin and diversity of tef (Vavilov, 1989).

Tef may have been domesticated in the highland of Ethiopia by the pre- Semitic people. But it is not certain for how long it was under their cultivation, Shaw, (1976) argued that tef might have been domesticated in Ethiopia before wheat and Barely were introduced; otherwise it would not have survived as a cultivated cereal. It is presumed that the crop was already under cultivation by the pre-Semitic people of Ethiopia when the Semitic invaded the Northern high land of Ethiopia between 1000 and 400B.C (Proteres, 1976).

2.1.3 Ecology and Agronomy of tef

Tef is adapted to a wide range of environments and is presently cultivated under diverse agro climatic conditions. It can be grown from sea level up to 2800 m above sea level (m.a.s.l) under various rainfalls, temperature and soil regimes. However, according to experience gained so far from national yield trial conducted at different location across the country, tef performs excellently at an altitude of 1800- 2100 m.a.s.l. Annual rainfall of 750-800mm, growing season rainfall of 450-550 mm and a temperature range of $10^{O}C$ - $27^{O}C$. A very good result can also be obtained at an altitude range of 1700-2200 m.a.s.l. and growing season rainfall of 300mm (Seifu, 1989).

In Ethiopia, tef is cultivated in much the same way as wheat and barley. Depending on the location and maturity period of the cultivar, it is grown during the main growing season

between July and November and also during the small rainy season between March and June. It is mainly cultivated as a mono crop, but occasionally under a multiple cropping system (Tavosoli, 1986).

2.1 4. The role of tef

The area devoted to tef cultivation is on the increase mainly due to the versality of the crop to the Ethiopian farmers. Some of the specific merits of tef that makes it important and preferred cereal by farmers are as follows:

1. The price for its grain and straw are higher compared to other cereals.
2. It performs better than other cereals (maize, sorghum) under low moisture stress condition; often it is a rescue crop as it survives and produces grain when planted after other cereals that have failed because of moisture shortage.
3. Tef also performs better than maize, wheat or sorghum in high moisture (water logged) conditions; flat and depressed lands that have a drainage problem are usually reserved for tef planting. Tef has a high recovery potential after being subjected to water logged conditions.
4. The grain can be stored in any kind of locally available material without being attacked by weevils.
5. No disease epidemic has threatened its performance and
6. The straw is a nutritious and highly preferred feed for cattle compared to other cereals (Seifu, 1989).

2.1.5. Status of tef in Ethiopia

Production

According to seven years (2001/01-2007/08) average data of the Central Statistical Authority, tef is annually cultivated on about 1.9 million hectares of land (Table 1), there by covering about 30.2% (largest) of the total coverage of cereals. Its area is expanding and showed a respective increase of 2.8%, 3.3%, 10.9% and 13.5%, from 1995/96 to 1998/99 relative to the preceding year. In 2005/06 the area increased by 15.7% as compared to 2006/07. The highest coverage was recorded in 2005/06 (2.2 million hectares), which was a favorable year with a record harvest in the country. Generally, because of a shortage of rainfall in some agro ecologies both in the Belg (short rainy season) and in the main rainy seasons, the area under cultivation for all cereal crops decreased by 15% in 2006/07. With an average of 1.6 million tons of grain per year, tef constitutes about 22% (second to maize, which is about 30%) of the gross yearly grain production of cereals (CSA, 2008).

Table 1 Area under cultivation, yield and production of tef from 2001/02- 2007/08

Year	Area (000ha)	Grain Yield (q*/ha)	Production (000q)
2001/02	1,385.7	9.8	13,559.7
2002/03	1,425.1	8.8	12,546.2
2003/04	1,899.0	9.8	18,582.0
2004/05	2,162.9	8.3	17,926.3
2005/06	2,238.7	9.1	20,371.7
2006/07	1,807.1	7.4	13,287.8
2007/08	2,091.3	7.9	16,422.9
Average	1,858.5	8.7	16,099.5

1q=100kg

Source: Central Statistics Authority (2006 and 2008)

According to the recent 2007/08 data, tef constitutes 31% of the land area devoted to seven cereal crops followed by maize (19.3%), sorghum (15.5%), wheat (14.6%) and barley (12.3%). In the same year, the share of tef production was 21.4% among the seven cereals surprassed by maize (31%) only.

Considering the country as a whole, tef is produced in seven regions to varying degrees (Table 2). Amhara and Oromia have the largest coverage of tef followed by Southern Nations Nationalities and Peoples Region (SNNPR) and Tigray. In the Amhara region, tef grows in 10 zones, most notably East Gojam, West Gojam, North Gonder, South Gonder, North Shewa and South Wello. Twelve zones grow tef in the Oromiya region, with major

production in West Shewa, East Welega, Jimma, East Shewa, North Shewa and West Welega. The major tef producing zones in the SNNPR are North Omo, Gurage, Hadiya, Kambata, Alaba -Tembaro and Keficho-Shekicho. In the Tigray region, the largest tef coverage is found in West, Central and South Tigray Zones.

Table2. Estimates of tef area by region for private peasant holdings in 2000/01

Region	Area Under cultivation (000 ha)	Per cent of the Total
Amhara	744.40	42.6
Oromia	729.53	41.7
SNNPR	123.24	7.1
Tigray	119.34	6.8
Beneshangul-Gumuz	22.93	1.3
Afar	4.66	0.3
Addis Ababa	2.99	0.2

Source: Central Statistics Authority (2007/08).

Economic importance

Price and contribution to the GDP

Price data collected from different wereda markets of East Shewa and North Shewa from 2001/02-2006/07 indicated that the prices of both the grain and the straw have increased steadily. The Debre Zeit Agricultural Research Center (DZARC) collected the prices from markets where farmers sell their produce directly to consumers. The grain price is an average of harvesting time and planting time data of white, mixed (sergegna) and red seeds. Average prices of the three classes of tef rose from 550 birr per quintal in 2001/02 to 1000

birr per quintal in 2006/07. Likewise, the straw price increased from 38 birr to 60 birr per quintal. Based on an average of four years DZARC collected price data, the grain of tef contributed 3.3 billion birr and the straw 1.7 billion birr to the gross domestic product (GDP) of the country. The total contribution is estimated to reach five billion birr. (CSA, 2008).

Using the national grain price collected by the Central Statistical Authority (2008) for the harvesting time of all zones of the country, the grain of tef contributed 2.34 billion birr to the GDP. This price figure does not include planting time price, which is normally higher than harvesting time, and therefore underestimates the real price. When the value of the straw is added, the figure rises to about four billion birr. It follows, therefore, that both the grain and straw of tef are estimated to contribute four to five billion birr per year to GDP of the country (CSA, 2008).

Export status

Official figures on the export status of tef have appeared during the past two years. Almost equal amounts (18,000 quintals) of tef grain were exported in 2006/07 and 2007/08, but the value obtained differed remarkably. About nine million birr was obtained in 2000/01 (550 birr/q) while 14.6 million birr was obtained in 2006/07 (950birr/q). 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 (CSA, 2000).

Yield potential

The six – year (2001/02 -2007/08) average national yield of tef was estimated to be 8.9 q/ha. However, farmers who use improved varieties and recommended management practices can easily get 17 -25 q/ha. Yield higher than 25 q/ha have been reported from several regions in the recent extension package activities. In experimental plots, tef yields up to 34q/ha in the presence of lodging that reduces yield by 17-25%. A study conducted under non –lodging conditions has demonstrated that yield can further be increased up to 46 q/ha. A record of 50 q/ha in some research plots has also been noted in the past (CSA, 2008).

2.1.6. Nutritional Status of tef

Tef is well known in Ethiopia for its nutritional quality. It provides about two-third of the daily dietary protein intake of most Ethiopians (Besrate *et al.*, 1980). Nutritionally, whole grain tef per 100g edible portion contain water 11.0gm Energy 357.6 Kcal, protein 9.6gm, carbohydrate 74.6gm, fat 2.53gm, fiber 2.63 gm, Thiamine 0.3mg, Riboflavin 0.3mg, Niacin 2.5 mg and ascorbic acid 88mg (EHNRI, 1998). It is an excellent source of essential amino acid, lysine that is most often deficient in grain foods. Tef contains more lysine than barely, millet and slightly less than rice and oats and it is glutten free (Janson *et al.*, 1962).

2.2 Fermentation

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

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

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

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

Over the centuries, fermentation has evolved and been refined and diversified. Today a variety of food products are derived from this technology in households, small scale food industries as well as in large enterprises. Furthermore, fermentation is an affordable food preservation technology and of economic importance to developing countries. It enhances the nutritional quality of foods and contributes to food safety where processing facilities are not available (Mosha and Vicen, 2004)

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

Chavan *et al.*, 2006). Fermented cereals porridges (and gels) are important staple food items for people of the West African sub region and are also important weaning foods for infants. Fermented foods having acidic pH are microbiologically safe and can be stored for a long time (Chauhan and khetaraul, 1989).

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

The microbial groups involved in spontaneous fermentation of cereal flour include *Lactobacillus spp, Acetobacter spp*, and *Saccharomyces cervisae*. Recently instead of the traditional lactic acid bacterial the use of probiotics bacteria such as Bifldobacteria to improve the therapeutic quality of food has gained considerable interest. Bifidobacteria are beneficial for human beings as they are the predominant member of the endogenous intestinal flora, capable of improving the balance of the intestinal microflora by preventing colonization of pathogens, activitating the immune system and increasing protein digestion (Kabeir *et al.*, 2004; Tsaoui *et al.*, 2008). A mean decrease of phytic acid in 64.8% after 96 hours and 39.0% after 72 hours in the fermentation of cereals grains were indicated in previous studies (Kaber *et al.*, 2002).

Organic acids produced, such as acetic, lactic, citric, formic and butyric acids, during fermentation potentiate zinc absorption by forming ligands with zinc (Andilid and Sandberg, 2002). Microbial fermentation enhances zinc bioavailability through hydrolysis induced by microbial phytase enzymes (Walingo, 2009). Fermentation of cereals reduces phytates in the diet could also favor enhanced absorption of other minerals like calcium and iron. Fermentation of cereals reduces phytate content via the action of phytase that catalyze conversion of phytate to inorganic orthophosphate and a series of myoinositiols, lower phosphoric esters of phytate (Walingo, 2009).

There are differences in optimal conditions for phytate degradation between plant species. Most cereal phytases have pH optima between 4-5 and 5-6 but pH optima of some legumes are neutral or alkaline. To optimize the food process for increased mineral bioavailability by phytate degradation, it is essential to know optimal conditions for the phytases, responsible for phytate degradation (Andlid and Sandberg 2002; Sandberg, 2002).

2.2.1 Fermentation of tef

Fermentation is a process with a potential for improving nutritional value of cereal grains. It has been reported that natural lactic acid fermentation improved the nutritive value of cereal. The moisture content, pH and titrable acidity (TTA) of the fermented dough and baked Enjera between 0 to 72 hr given below;

Table 3. Change in moisture content, pH and titrable acidity during fermentation and baking

Time (h)	Moisture content %	pH	Titrable acidity
0	65.3± 0.32	6.3± 0.05	37±0.2
24	64.9± 0.33	5.8± 0.14	42±1.04
48	65.6± 0.44	43± 0.1	82±0.82
72	64.9 ± 1.16	3.9± 0.05	90±0.22
Enjera	58.9 ± 1.33	4.0± 0.05	60±1.4

Source: Melaku (2005)

Moisture content differs negligible between 0 and 72h of fermentation. However, there was an increase by about 8% during formation of Enjera due to further addition of water to make a thin paste.

The pH value fall considerably from 6.3 to 3.9 as fermentation progressed. Microorganisms that initiate the fermentation process are capable of lowering the initial pH. Furthermore, the environmental conditions such as the pH and moisture content as well as starch hydrolyzing bacteria and increased the amount of fermentable sugars, which would concomitantly increase acid production and further decrease in pH. Similar pattern of fall in pH has also been reported during fermentation of cereals. Parallel to this fall in pH, the titrable acidity increased during fermentation but decreased in the baked product mainly due to the loss of acetic acid (Melaku *et al.*, 2005).

2.2.2. Fermentation process

Ordinarily the grain is cleaned first by sifting and then ground into flour; studies have shown that the milling, tef gives a return of 99% where as wheat gives 60-80% (Taddesse, 1975).

Sequence of events occurring in the fermentation of tef is as follows (Berhanu *et al.*, 1982).

0 hours

Initiation of fermentation (pH 6.5-7.5)

24 hours

Dough rises, gas evolution become vigorous; Engera could be baked anytime here after, PH 5-5.5

31 hours

Dough begins to settle, gas evolution decreases initial liquid/solid separation begins, pH 4.7-5.0

48 hours

Dough settles completely, gas evolution is at its minimum level, complete liquid/solid separation is achieved, pH 5.8-4.2

72 hours

Liquid layer becomes yellow in color film yeast form confluent growth on the surface of the liquid, pH 3.5-3.8

2.3 Baking

Baking is the cooking of food by dry heat in an oven in which the action of the dry convection heat is modified by steam (Egli, 2001). There are three methods of baking can be identified.

1. Dry baking: when baking, steam rises from the water content of the food, this steam combines with the dry heat of the oven to cook the food.

2. Increased humidity baking: When baking certain foods example bread the oven humidity is increased by placing a bowl of water or injection steam into the oven, thus increase the water content of the food and so improve the eating quality.

3. Bain marie: when baking takes place food in a container of water (bain marie) which modifies the heat so that the food cooks more slowly, doesn't over heat or over cook (Boriso and Laiyi, 1967). Baking process has the following advantage.

1. A wide range of sweet foods can be produced

2. Bakery products yield appetizing goods with eye-appeal and mouth-watering aromas.

3. Bulk cooking can be achieved with uniformity of colour and degree of cooking.

4. Baking ovens have effective manual or automatic controls.

5. These is straightforward access for loading and removal of items (Egli, 2001) and baking has the following disadvantages.

1. Require regular attention.

2. Ovens are expensive to heat.

2.3.1. Baking process of Engera

Baking to form `` Engera`` can be summarize by the flow diagram (figure 1) as follows.

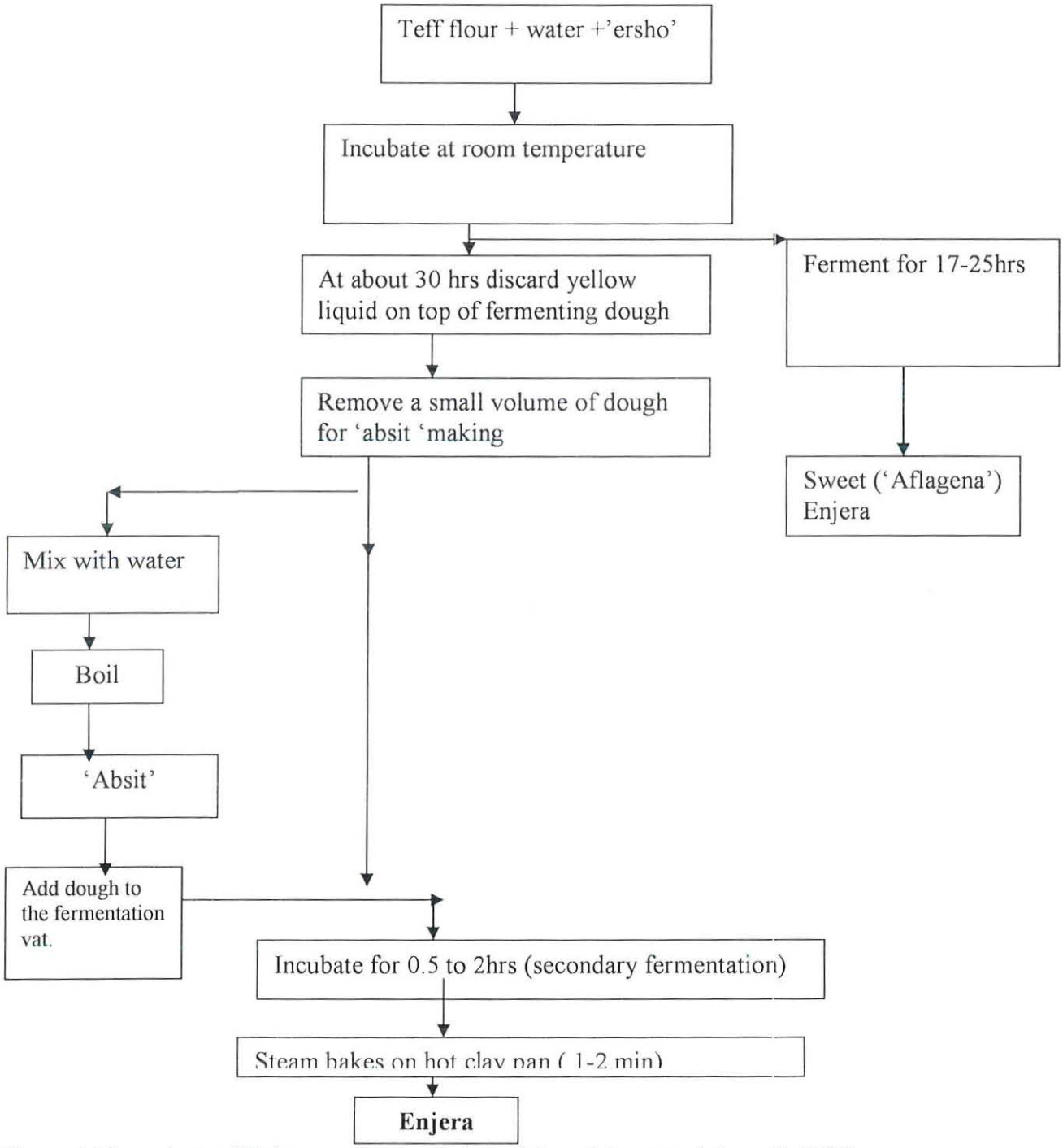

Figure.1 Flow chart of Enjera production (adapted from Mogesse Ashenafi, 2006).

2.4 Micronutrients in tef

Tef is a cereal which contains different micronutrients within it such as calcium, iron, zinc, etc (Agren and Gibson, 1968). Micronutrients are essential for the optimal human growth and development, and healthy maintenance of body over a life span. Micronutrients are minerals needed (but not synthesized) and vitamins in small amounts for a wide range of functions and processes (Ruel, 2001).

Globally, micronutrients deficiency affects more than two billon people. Although less prevalent in higher-income populations, these deficiencies do occur in such groups, especially among premature infants, children and elderly (Ruel, 2001).

Micronutrients are required to fight against diseases, bear healthy children and to maintain strong bodies and mental sharpness. Micronutrients deficiency can cause mental retardation, decrease immunity, low risk capacity, blindness, premature death, and learning disability. They affect adult productivity, educational achievement, women health, children survival and overall resistance to illness. They may impaire immune function; increase the risk of opportunistic infections, and the severity of disease (Gibson and Donovan 1998).

2.4.1 Iron

Ethiopia may represent the country with the highest intake in the world of iron per person per day, even greater than that of the Bantu natives in South Africa, an average iron intake per person per day of 471mg with a range of 98-141mg. Dietary survey and food analysis

performed revealed the following as regard to iron intake. An adult man eats about 2-3 Enjerra per days, each baked of 200-250g tef flour had a mean iron intake of 520mg daily with a range of 250-870 mg (Hafvander, 1990).

Iron is the fourth most abundant and one of the cheapest elements in the Earth's crust but iron deficiency is still the most prevalent nutritional disorder in the world (Lind, 2004)
The iron in tef used in the synthesis of hemoglobin plays a vital role in the transport of oxygen, and myoglobin (Besrat *et al.*1980). It contributes to the formation of iron-containing enzymes that are important for energy production, immune defense and thyroid function. Iron has a role in healthy physical growth; reproductive outcomes, in cognitive performance and the immune system (Kayode, 2006).

The main factors of iron deficiency are poor iron content of the diet, low bioavailability of iron in the diet, or both. Food components such as phytate, tannins, and selected dietary fibers, which bind iron in the intestinal lumen, can impair iron absorption. Phytate has the greatest effect on iron status because many plant foods have high phytate content that can severely impair iron absorption (Browen and Mendoza, 2001).

Tef is often high in iron content and contains significant quantities of iron absorption inhibitors, phytic acid and condensed tannins (Kayode, 2006). The absorption of iron in food depends on iron status of the body; the presence of iron absorption inhibitors and

enhancers, types of iron (haem and non- haem) vitamin A status of the body and the status of other micronutrients (Pynaeret *et al.*, 2006).

2.4.2. Zinc

Cases of zinc deficiency in human were first described as early as the 1960s in male adolescence in the Middle East (Gibson and Donovan 1998). Since that time zinc deficiency has been identified in many other regions of the world, including Ethiopia (Melaku *et al.*, 2005).

Zinc is the fourth important micronutrient after iodine, iron and vitamin A. Zinc obtained from foods is found in all body fluids, tissues and organs, especially the skeletal muscles and the bones (Mertez,1992). Zinc plays vital role in growth, protein synthesis, cell division which makes adolescents, children and infants and pregnant women at risk for inadequate zinc intake. Zinc contributes to immune system functioning, appetite, night vision, taste, growth and the reproduction. Zinc also contributes to stabilize cellular components and the structure of membranes (Hambidge, 1987). Zinc is an essential component of a large number of enzymes involved in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids and in the metabolism of other micronutrients (Prashd and Shnkar, 1998).

Deficiency of zinc is associated with immune function; increased susceptibility to the severity of infections, poor growth, and adverse outcomes of pregnancy and neuro behavioral abnormalities. Zinc deficiency is due to the low consumption of animal source

foods, which are rich in zinc, and a high intake of cereals and legumes, which contain substantial amount of phytate (Sandberg, 2002).

The bioavailability of zinc in plant – based foods is reduced by the phytate, fiber, calcium and lignin present in the plant matrix. Specific food processing techniques such as soaking, germination and fermentation also help to reduce the impact of zinc inhibitors (Gibsion *et al.*, 1997).

2.4.3 Calcium

Calcium is the most abundant mineral element in the body. Calcium in tef plays an important role for structure and strength of bones. Calcium (small proportion) regulates critical function including activity of enzymes, muscle contraction and nerve impulses. The element is present in two body parts; bone and teeth. The body of an adult contains about 1.2 kg calcium, accounting for about 2% of body weight. Sufficient calcium intake is essential for obtaining optimal peak bone mass in youth and minimizing bone loose later in life (Gurr, 1999).

The proportion of calcium absorbed from food depends on how the calcium is bound chemically in the food and the presence of many substances also present in the food, which may either enhance or inhibit absorption (Fernandez and Pawloski, 2001).

2.4.4 Mineral status of tef

Besides providing protein and calories, tef is a good source of minerals particularly irons (Harold and Leslie, 2002). Compared with other cereals, tef is also an excellent source of zinc and has many times amount of calcium. White tef contain 160mg and red tef contain 162mg per composition of 100g calcium (Tadesse, 1975). Potassium, zinc 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 Mengesha, 1966. according to some studies Besrat *et al.*,1980; Areda *et al.*,1993, is attributed to extraneous contaminants it receive from soil during threshing and not due to high intrinsic grain content. Whether derived from contamination or from inherent grain contents, however, the higher iron intake from consumption of tef than non-tef consumers is unequivocal (Mengesha, 1996). This can be conformed from the fact that non-tef consumers have a lower level of hemoglobin and hook worm anemia develops in non-tef eaters if they are infested with hook worm. On the other hand, since tef eaters have higher levels of hemoglobin in their blood, they don't suffer from hook worm anemia even when infested (Areda *et al.*, 1993). Moreover, consuming tef is reported to prevent anemia related to pregnancy. When tef is used to make Enjera, a short fermentation process allows the yeast to generate more vitamins (Mengesha, 1966). Fermentation of tef flour decrease the pH of the dough with partial break down of soluble starch. This also increases the dialyzable portion of its Fe, P and Zn contents. The increases in dialyzable Fe results inhance the bioavailability (Areda *et al.*, 1993).The fermentation process increase the bioavailability of metals. Hence reduces iron deficiency

anemia, among tef-Enjera consuming peoples However, a long duration of fermentation decrease the protein quality of Enjera.

2.5. Antinutritional factors in tef

Antinutritional compounds (such as phytate and tannin etc) are plant constituents which play an important role in biological function of plants. These compounds reduce digestibility of nutrients and absorption of minerals in human (Dicko, 2005).

2.5.1 Phytic acid

Phytic acid is a major storage form of phosphorous and the source of inositols in grains. Approximately 75% of a mature seed total phosphorous is found as a single compound called phytic acid formally referred to as myoinsitol 1,2,3,4,5,6,-hexakis dihydrogenphosphate. The biochemical behavior and the nutritional value of phytic acid are governed by the strong chelating ability of the molecule (Dicko 2005; Melaku *et al.*, 2005, Urbano *et.al*, 2005). Phytic acid forms complexes with proteins, starch and lipids and decrease their digestibility. It inhibits the action of some enzymes as amylase, acid phosphotase and trypsin, having an adverse influence upon the digestive process (Allen and Ahluwalia, 1997; Greiner *et al.*, 2006). In human, phytic acid is a chelating agent, which binds nutritionally important mono- and divalent minerals (i.e. Ca^{+2}, Zn^{+2}, Fe^{+2}, Mg^{+2} and Co^{+2}) and forms complex phytate. This antinutritional factor chelate dietary minerals in the gastrointestinal tract reducing their bioavailability and bioaccessibility (Hurrel and Reddy 2003; Dicko, 2005).

Phytic acid forms complex compounds with cations such as zinc, magnesium and calcium at physiological pH. Phytates are complex compounds formed by phytic acid and cations. These complexes are insoluble or difficult to hydrolyze during digestion. Phytic acid inhibit absorption of minerals such as iron, calcium and, zinc by humans and other monogastric animals in a dose-dependent manner. When the molar of phytic: iron and phytic: zinc is above 0.15 and 1.5 respectively, phytate inhibits absorption of the respective minerals (Allen and Ahluwalia, 1997; Melaku *et al., 2005*).

Phytate in red tef constituted more than 55% of total phosphorous; this value was 58% for white tef. Red tef seeds also contained 12% higher total phosphorous compared to white tef seeds. The inorganic phosphorous content of red tef seeds was 37% higher than that of white seeds. However, iron, calcium, zinc and phytate concentrations as well as phytate: Zinc molar ratios were similar in both tef varieties (Kalbesa Urega and Narashima, 1998).

2.5.2 Tannins

Oligomers of flavan-3-ols and flavan-3, 4-diols called condensed tannins occurs widely in cereals and legumes (Haard, 1996). These compounds are concentrated in the bran fraction of cereals. Tannin-protein complexes can cause inactivation of digestive enzymes and reduce protein digestability by interaction of protein substrate with ionizable iron. The presence of tannins in food can therefore lower feed efficiency, depress growth, decrease iron absorption, damage the mucosal lining of the gastrointestinal tract, alter execration of

cations and increase execration of protein and essential amino acids (Reddy *et al.*,1985; Hassen,2007).

Condensed tannins, also known as proanthocyanidians, are high-molecular weight polyphenols. Tannin inhibits the activity of some enzymes and therefore adversely influence protein digest ability and cellulose breakdown. The acidic hydrolysis of tannin leads to the generation of glucose and gallic acid. The galloyl configuration binds with minerals through its adjacent hydroxyl groups. The derived iron-galloyl complex is insoluble (Asante, 1995; Egli, 2001).

2.6. Interactions between phytic acid and minerals

Phytic acid (myo-inositol- 1, 2, 3, 4, 5, 6- hexakis phosphate) is the major phosphorous storage compound in plant seeds and can account for up to 80% of seed total phosphorous. The remaining phosphorous is represented by soluble inorganic phosphate and cellular phosphorous (Phosphorous bound in nucleic acids, phosporylated proteins, phosphorous-lipids, phosphorous- sugars). Because of its high density of negatively charged phosphate group, Phytate forms mixed salts with mineral cations which are assumed to play an important role in mineral storage. These salts called phytins predominantly contain K and Mg. whereas, other metals such as Ca, Zn, Fe or Cu are found in much smaller amount (Odell *et al.*, 1999; Kamar et *al.*, 2002).

2.6.1 Calcium and phytic acid

The factors controlling bioavailability of calcium can be divided into intrinsic and extrinsic factors. Intrinsic factors are age, sex, pregnancy and lactation. Extrinsic factors include a number of dietary variables that may influence calcium absorption, such as amount of calcium, vitamin D, oxalate, phosphopeptides, fat, lactose and phytic acid and a number of dietary variables that may influence urinary calcium excretion such as salt, phosphorus, protein, alcohol and caffeine (Guegven and Poiniollarh, 2000).

In human, phytic acid decreases calcium absorption and phytic break down improves calcium availability. If calcium assimilation can be reduced by phytic acid, a high calcium phytic acid molar ratio in food leads to the absence of hydrolysis products in the intestine. The reason for the decreased phytic acid degradation caused by calcium may be the formation of insoluble calcium phytate complexes which are poor substrates for phytase (Miyazawa, 1996; Reinhold, 1999). The ingestion of phytic-rich foods maintains adequate calcium urinary levels to permit effective crystallization inhibition of calcium salts and consequently prevent renal stone development (Grases *et al.*, 2000; Duhan *et al.*, 2002).

2.6.2 Iron and phytic acid

The single most prevalent deficiency on a world wide scale is iron deficiency anemia, affecting an estimated 30% of the world's population in Europe. Iron deficiency is considered to be of the main nutritional deficiency disorders (Hereberg *et al.*, 2001). Iron deficiencies are caused by insufficient intakes of iron, increased iron requirement of pregnancies or by blood loss or impaired absorption. The source of iron as well as composition of meal is of great importance because dietary factors play an important role in iron absorption.

There are two kinds of dietary iron: haem iron and non-haem iron. Haem iron is poorly affected by other components in the diets. Non –haem iron comprises the main part of the iron intake absorbed in ionic form by receptors on the mucosa cells and its bioavailability varies depending on iron status of the subjects and different dietary factors. There is evidence that phytic acid has a very marked inhibitory effect on the absorption of non-haem iron in human (Gillooly and Bothwall, 1983). Phytic acid decreases iron solubility and the inhibition of iron absorption is closely related to the content of phytate in bread (Sandberg, 2002). The inhibitory effects of Phytic acid on iron can be counter acted by iron absorption enhancers such as protein or organic acids. Ascorbic acid is the most effective enhancers of non-hame iron absorption (Broune and Rossander, 1989; Reddy *et al.*, 1996).

2.6.3 Zinc and phytic acid

Zinc is an essential trace element involved in the immune function, in the activation of many enzymes and in the growth. However, Zn deficiency has been recognized in western countries due to inadequate dietary supply, abnormal blood losses or high physiological requirements for growth, puberty, pregnancy and lactation (Hereberg *et al.*, 2001).

The availability of Zn for intestinal absorption and body utilization is the net effect of absorption inhibiting and promoting components of the diet. The amount of Phytic acid, the type and amount of protein and the total Zn content have a major impact on the amount of Zn absorbed from foods under normal physiologic conditions. Factors affecting the amount of Zn available in intestinal absorption determine Zn bioavailability. Certain types of diets could alter the reabsorption of endogenous intestinally excreted Zn and thereby affect utilization (Pabon and Forbes 2001).

Phytic acid is the major determinant of Zn absorption, especially for diets with a low animal protein content. Phytic acid strongly binds Zn in the gastrointestinal tract and reduces its availability for absorption and reabsorption (Flnagan. 1990). The inhibitory effects of Phytic acid on Zn can be predicted by the molar ratios of Phytic acid-to-Zn in the diet. When the dietary Zn intake is close to the requirement, molar ratios in excess of 15:1 progressively inhibit Zn absorption and have been associated with suboptimal Zn status in

humans (Gibson *et al.*, 1997).Furthermore, high levels of Ca exacerbate the inhibitory effect of Phytic acid on Zn absorption in humans by forming a Ca – Zn- Phytic acid complex in the intestine that is even less soluble than phyate complex formed in either ion alone, hence. Phytic acid x Ca/ Zn ratios may be better predicators of Zn bioavailability than Phytic acid /Zn molar ratios alone (Fordyce *et al.*, 1992).

In order to increase Zn bioavailability. Foods can be improved by I. increasing the total amount of dietary Zn. However fractional Zn absorption is dependent on the Zn content of the diet. At low Zn content with no potential inhibitory agents, fractional absorption can be as high as >50%. High Zn intakes result in a low absorption percent, because of the saturation of the active transport mechanisms for Zn. II. Enhancing the bioavailability of Zn consumed. This is possible by promoting the intake of enhancers and reducing the impact of Phytic acid on intestinal Zn absorption.

Food interactions are also determinants for Zn absorption (Lonnerdal, 2000). Dietary proteins can potentially facilitate Zn absorption even in the presence of Phytic acid. Proteins prevent the precipitation of Zn in the intestinal lumen and amino acids such as cysteine or peptides which facilitate Zn uptake by the mucosal cells (Sandsrom and Robins 2002). Consuming fermented foods leads to enhanced Zn absorption. This effect is attributed to presence of organic acids: acetic, citric, lactic or mallic acids which form soluble ligands

with Zn, thereby preventing the formation of insoluble phyates.The solubility of Zn at the site of absorption probably has a major impact on its availability in foods as relatively easily solubilized at gastric P^{H}. whereas it binds to organic components at higher pH. Thus small molecular weight ligands, such as organic acids have the potential to increase solubility and facilitate absorption of Zn. (Pabon and Forbes, 2001).

3. Materials and methods

3.1 Materials

Samples of the two varieties white tef variety (DZ-Cr-387, Qunicho) and red tef Varity (DZ-01-99) were collected from Debere Zeit Agricultural Research Center (DZARC). After the sample was collected. It was packed in polyethylene bags and transported to Food Science Laboratory; AAU. These two Varieties are currently registered as new varieties of tef in Ethiopia. Except the genetic information, area of production, season of production, production per hectare, etc. have been documented for these two varieties. However, no information is available about the nutritional components of these two varieties.

3.1.1. Agronomic and morphological characters of samples

3.1.1.1. Variety Dz –Cr-387[white tef, Quincho]

- Adaptation area: DZRAC
- Altitude: 1850-2400m.a.s.l
- Rainfall (mm): 1000-1200
- Seed rate (kg/ha): 25-30
- Planting date: July
- Fertilizer rate (kg/ha): 100 DAP
- Days to heading: 500 - 577
- Days to maturity: 110—134days.
- Panicle length (cm): 31-44
- Panicle form: Fairly loose
- Plant height (cm): 70-110
- Growth habit: Erect but highly bending at maturity

- 1000 seed weight (g): 3-4
- Flower color: Green
- Seed color: Very white
- Crop pest reaction : Tolerant to diseases and pests
- Yield (q/ha):

  Research field: 15-27

  Farmers' field: 13-23
- Year of release: 2008
- . Breeder maintainer: DZRAC

3.1.1.2. Variety DZ - 01-99 (Red Tef)

- Adaptation area: DZRAC similar agro ecology
- Altitude: 1800-2600 m.a.s.l
- Rain fall/ average(mm): 1230-1500
- Planting date: Early July or late July
- Fertilizer rate:

  80/120kg/ ha for black soil.

  40/60kg/ ha for red soil.
- Seed rate(kg/ha) 25-30
- Days to maturity (days): 92-127
- Growth habit: Erect
- Plant height (cm): 107
- Seed color: Red
- Flower lema color: Red
- Crop pest reaction: No significant pest was observed.
- Yield
  - Research station(q/ha): 19-27
  - Farmers' field (q/ha): 16-22

- Year of release: 2006.
    - Breeder/Maintainer: DZRAC (source: Crop variety Register .MoARD animal and plant Health Regulatory Directorate)

3.1.3. Equipments, Chemicals and Glass wares.

All chemicals used for analysis were AR grade and obtained sigma Aldrich Company. (Analytically grade) Similarly, the glass wares were cleaned and free from any possible contamination prior to analysis.

3.1.4 Sample preparation

All the Samples were cleaned manually to remove foreign matters (Winnowing,)

A. Preparation of the flour

The seeds were ground to coarse flour in an electrical mill and passing through 0.452mm screen.

B, Tef Fermentation

Fermentation was carried out in triplicate by mixing thoroughly tef flour with distilled water (1:1.8w/v ratio).The homogenous slurry was allowed to ferment at 20-23^{O}C in a large, round, glass jar (fermentation vat) after fermentation for 72 hr. Triplicate samples for

analysis were oven-dried at 90°C for 20hr, ground to fine powder with mortar and pestil and kept for analysis.

c. Preparation of the dough.

To prepare the dough the following steps were used:-

1. The flour sifted into a container that is large to hold the entire.
2. The *ersho* was added to the dough and then mixed using a large stirrer. (The *ersho* was prepared from the flour without mixing with other samples).
3. Three liters of distilled water were added and mixed with the dough very well.
4. Three more liters of distilled water were added gradually along with frequent stirring all the time until it is mixed well.
5. The dough was covered and allows it to ferment for three days (72hr).
6. The dough is oven dried at 90^{O}C for 20 hr.

d. Baking the Enjerra

For preparation of Enjerra the following steps were used:-

1. The water was decanted from the dough.
2. 100g of dough was added to a liter of distilled water and then boiled it.
3. Three liters of cold distilled water were mixed with the dough and stirred well, and allowed to stand for 30 minutes.
4. In the mean time, the stove is heated (an oven for baking the Enjera).
5. To bake Enjerra, the batter was poured on to the hot greased stove, following a circular motion from the center towards the edge of the stove to make a round Enjera. When the

Enjera began to form numerous small eyes, cover the stove with thin-made lid and leave it for 1-2minutes. Use about a half liter of batter for each Enjera.

6. The baked Enjera was oven dried at 90^0 C for 20hr.

3.2 Methods

3.2.1 Laboratory analysis

Each laboratory determination was carried out on two separate fresh samples (in Triplicate).

3.2.2 Determination of moisture content (AOAC 925.09, 2000).

The aluminum dishes used for the moisture determination were dried at 130^{O}C for 1hr using drying oven (DHG-9055A). The dishes were removed and kept in desiccators for about 30 minutes. The weight of empty dish was measured as M_1. This was regulated until constant weight was obtained. About 5gm of the sample was weighed using analytical balance (Model-LA-204) in to the dish and recorded as M_2. The sample was mixed thoroughly and dried at 100 ^{O}C for 6hrs. And kept in desiccators to cool. After cooling the weight was taken as M_3. And kept in oven for another 15 minutes. Then after cooling in the desiccators the sample was weighed. This process was repeated until constant weight was obtained (AOAC, 2000). Then, the moisture content was calculated using the following formulae:-

Moisture (%) = $\frac{M_2-M_3}{M_2-M_1}$ x 100

M_1= Weight of the dish

M_2= Weight of the dish and the sample before drying

M_3 = Weight of the dish and the sample after drying.

3.2.3 Determination of total ash content (AOAC 923.03, 2000).

Porcelain dishes were placed in a muffle furnace (Carbolite, Aston, LaneHope, Sheffield s30 2RR. England) for 30 min at 550^{O}C. The dishes were cooled in desiccators (with granular silica gel) for about 30 minutes and weighed to the nearest milligram. About 2.5g of fresh sample (in triplicate) was placed in dish. Dishes were placed on a hot plate under a fume-hood and the temperature was slowly increased until smoking ceases and the samples became thoroughly charred. The dishes with sample were placed inside the muffle furnace at 550^{O}C for 5 hr and cooled in desiccators for 1 hr. The ash was clean and white in appearance. When cooled to room temperature, each dish with ash was reweighed to the nearest milligram (Osborne and Vooget, 1978; AOAC, 2005).

% Ash (wet basis) = $\frac{M_{ASH}}{M_{WET}}$ x100

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

3.2.4. Determination of crude protein content. (AOAC 979.09.2000).

Digestion: About 0.5 g of fresh samples (in Triplicate) was taken in a tecator tube and 6m1 of acid mixture (and 100 parts of concentrated sulfuric acid) 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 rack. Three gram of catalyst mixture (ground 0.5g of Copper sulfate with 100g of potassium sulfate) were added into each tube, and allowed to stand for about 10 minutes before digestion. When the temperature of the digester attains $370^{O}C$ the tubes were lowered into the digester. The digestion was continued to about 5hrs until a clear solution was obtained. The tubes in the rack were cooled in a fume hood; 15ml of deionized 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 sulfuric acid to reddish color using Kjeldehal apparatus (Tectar, 1979; AOAC, 2000).

mg nitrogen in the sample	= V x N x 14
g nitrogen /100g sample	= mg of nitrogen x 100/mg sample
Total nitrogen (%)	= $[(V - V_b) \times N \times 1.4)/W$
Crude protein (%)	= total nitrogen (%) x 6.25

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

3.2.5. Determination of crude fat content (AOAC 4.5.01, 2000).

The flasks were washed and then dried in drying oven at 92^{O}C for 1hr and cooled in desiccators. The masses of the cooled flasks were measured by analytical balance and recorded as M_1. About 2 gm of the powdered sample was weighed and placed in to each thimble lined with cotton at their bottom. The thimbles with their sample were placed in to the Soxhletlet Extraction Apparatus (Soxhlet extraction unit 2055 soxtecextraction unit, Foss extractor, Sweden). Then 50 ml of diethyl ether were added in to each flask and the extraction process was done for about 3 hrs. Then the flask was placed in drying oven at 92^{O}C for 1hr. The flasks with their contents were then placed in desiccators for 30 minutes. The weight of each flask with its fat contents was measured as M_2. Then the total lipid amount was calculated using the following formula. (Tecator, 1979; AOAC, 2005).

Lipid (%) = $(M_2 - M_1) / M \times 100$

M_1 = Weight of dried flask.

M_2= Weight of flask and lipid extracted.

M = weight of sample on dry basis.

3.2.6 Determination of crude fiber content (AOAC 962.09, 2000).

Digestion: About 1.7000g of dried fresh sample (in triplicate) was placed into a 600 ml of beaker; 200ml of 1.25% molar 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 minutes of heating, 20ml of 28% molar KOH was added and boiled gently for further 30 minutes, 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, the wall of the beaker was rinsed with hot distilled water several times; and 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% molar H_2SO_4 and filtered, and then washed with hot distilled water and again filtered; and washed with 1% molar NaOH and filtered. The residue was washed with hot distilled water and filtered; and again washed with 1% molar H_2SO_4 and filtered. Finally the residue was washed with water and acetone.

Drying and combustion: The crucible with its content was dried in a drying oven for 2hr at 130^{O}C and cooled for 30 min in a desiccators (with granular silica gel), and then weighed recorded as W_1). The crucible was transferred to muffle furnace and heated for 30 min at 550^{O}C and the crucible was cooled in a desiccators and weighed (recorded as W_2) (AOAC, 2005).

Crude fiber g/100g= $[(W_2)*100]/W_3$

W_1= weight of crucible with sample after drying; W_2 = weight of crucible with sample after ashing; W_3= fresh sample weight.

3.2.7 Determination of total carbohydrate content

Total carbohydrate content was estimated by the difference, as percentage (%) on wet basis (EHNRI, 1998)

Carbohydrate (%) = 100-(fat % + protein%+ ash% +moisture%+

3.2.8 Determination of gross energy content

The energy values of the formulations were determined by computation and expressed In kilo Calories. It was calculated from fat, carbohydrate and protein contents using the

Kcal/100g= (4xcarbohydrate) + (4xproteing) + (9xfat)	1kJ/100g=4.18 kcal/100g

Atwater's conversion factors: 1 g fat= 9 kcal, 1g protein=4 kcal and 1 g carbohydrate=4 kcal (EHNRI, 1998)

3.2.9 Determination of minerals

Ash was obtained from dry ashing of food samples (3.2.3). The ash was wetted completely with 5ml of 6N HC1, and dried on a low temperature hot plate. Seven ml of 3N HC1 was added to the dried ash and heated on the hot plate until the solution just boils. The ash solution was cooled to room temperature in a hood and filtered into a 50 ml graduated flask using a filter paper (Whatman 42,125mm). Five ml of 3N HCl was added into each crucible dishes and heated until the solution just boils, cooled, and filtered into the flask. The crucible dishes were again washed three times with deionized water; the washings were filtered into the flask. A 2.5 ml of 10% lanthanum chloride solution was added into each graduated flask. Then, the solution was cooled and diluted to 50 ml with deionized water.

A blank which contained 12ml 3N HCl and deionized water in 50 volumetric flask was also prepared.

Standard solutions: Four series of working standard metal solutions (Table 3)were prepared by appropriate dilution of the metal stock solutions (nitrate of the metal) with deionized water containing 2.4 ml 3N HCl in 10ml volumetric flask. After manipulating the instrument operation procedure, calibration graph (concentration versus absorbance) for each element using the prepared standard solutions was prepared using AAS.

The sample concentrations were analyzed using flame atomic absorption spectrophotometer by aspirating deionized water. Sample blank solution was run with the sample solution. A single mineral hollow cathode lamp was used for each element (Osborne and Voogt, 1978).

Table 3. Series of working standard solutions for mineral determination

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

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

W=weight (g) of samples; V=50ml= (Volume (V) of extract)

a = Concentration (mg/ml) of sample solution b=concentration (mg/ml) of blank solution

3.2.10. Determination of phytate content

About 0.1500g of fresh samples was extracted with 10ml 2.4% HCl in a mechanical shaker for 1hour at a room temperature. The extract was centrifuged (Dyhac centrifuge clay Adams Bacton, Dikinson and company USA.) at 3000 rpm for 30min. The clear supernatant was used for phytate estimation. One ml Wade of reagent (containing 0.03% solution of $FeCl_3$ $6H_2O$ and 0.3% of sulfosalicilic acid in water) was added to 3ml of the sample solution (supernatant) and the mixture was mixed on a vortex for 5 seconds. The absorbance of the sample solutions were measured at 500 nm using UV-VIS spectrophotometer (Buck scientific Atomic absorption spectrometer Zloup Canada).

A series of standard solutions were prepared containing 0, 5, 10, 20 and 40µg /ml of phytic acid (analytical grade sodium phytate) in 2.4% HCl. Three ml of the standard solution was added into 15ml of centrifuge tubes. Three ml of water were prepared to serve as standard blank. One ml of the Wade reagent was added to each test tube and the solution was mixed on a vortex mixer for 5 seconds. The mixture was centrifuged for 10 minutes and the absorbance of the solutions (both the sample and standard) was measured at 500nm by using deionized water as sample blank (Latta and Eskin, 1980)

Phytic acid in mg/100g= {[(absorbance-intercept) / (slope*density*weight of sample)]*10}/3

3.2.11 Determination of condensed tannin

About 2.0g of fresh sample was weighed and transferred to screw cap test tubes (in triplicate). The samples were extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with a mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000 rpm for 5 minutes. One ml of supernatant was taken and mixed with 5 ml of Vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol).

D-catechin was used as standard for condensed tannin determination..Forty mg of D-catechin were weighed and dissolved in 1000 ml of 1% HCl in methanol, which was used as stock solution. Exactly 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tubes and the volume of each test tube was adjusted to 1.0 ml with 1% HCl in methanol. Five ml of vanillin-HCl reagent was added into each test tube.

After 20 minutes, the absorbance of the solutions and the standard solution were measured at 500 nm by using deionized water as blank, and the calibration curve was constructed from a series of standard solution using SPSS version 15. Concentration of tannin was read in mg of D-catechin per gm of sample (Maxson and Rooney, 1972).

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

3.2.12 Determination of total phosphorous

The sample solutions prepared for mineral determination was used for phosphorous determination. One ml of the clear extract (sample solution prepared for mineral determination) was diluted into 100 ml with deionized water. Five ml of the sample solution were added into test tubes. Exactly 0.5 ml of molybdate and 0.2 ml amino naphtholsuphonic acid were added into the test tubes (sample solution) and mixed thoroughly step by step. The solution was allowed to stand for 10 minute

Standard solution; Six series of working standard phosphorous solutions (0.2, 0.4, 0.6, 0.8 1.0 and 1.2 mg/ml) were prepared by appropriate dilution of the phosphorous stock solution (1000mg /ml of KH_2PO_4) with deionized water using 10ml volumetric flask. After manipulating the instrument operation procedure, the absorbance (A) of the sample solution was measured at 660 nm against distilled water using UV- VIS spectrophotometer (Buck scientific Atomic absorption spectrometer Zloup Canada). The standard and sample blank solutions were run with the sample. Calibration graph (concentration versus absorbance) for each element using the prepared standard solutions was prepared (Fiska and Subboarro, 1999).

$$\text{Phosphorous content (mg/100g)} = \frac{(\text{sample absorbance - blank absorbance})}{(\text{Slope} * \text{Wt.Sample})} * \text{dilution factor}$$

Non –phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus content (mg/100g) (Duhan *et al.*, 2002).

Phytate phosphorus (mg/100g) = (phytate x 28.18) /100

3.3 Data management and statistical analysis

Each determination was carried out on three separate samples and analyzed in triplicate. Results were reported as an averaged value (mean $\pm$ standard deviation). Data was analyzed by one-way analysis of variance (ANOVA) model using SPSS version 15.0 .soft ware. Differences among treatments were determined by the Duncun multiple comparison tests Statistical significance test was set at $P < 0.05$.

4. Results and Discussion

4.1 Effect of Processing on the proximate composition of tef.

4.1.1. Moisture content

There was no significant difference ($p > 0.05$) on each processing methods Table 5. The results indicated that, the maximum content was observed at 72 hr processing being 9.05% and 8.60% fermented and baked samples of white tef (DZ-Cr-387) and 8.95% and 8.84% of fermented and baked of red tef (DZ-01-99). However, the relative increase in moisture content may be attributed due to a variation in the treatment during the drying processes of the samples. The moisture content of cereal standard percentage recommended is 5-10% (Olorunfemi *et al., 2006*). while the maximum moisture contents attained during the 72h of fermentation of tef were 8.60 % and 8.84 %. Thus fermentation has no overall effect on the standard percentage of moisture content and an increasing trend might be due to the further addition of water to make a thin paste (Alemayehu, 1990). When we compare the results of the current study with that of the previous local research of white and red tef the following observation have been recorded 8.8% and 8.4% of fermented and baked white tef and 8.6% and 8.2% of fermented and baked red tef (Experimental Station Bulletin 66, 1969), and the moisture content in the present study of tef was similar to the studies under taken by (EHNRI, 1998; Yewelsew *et al.*, 2007).

Table 5:- Effect of processing on proximate composition of tef

Sample	Moisture %	Total Ash %	Crude Protein %	Crude Fat %	Crude Fiber %	Crude Carbohydrate %	Energy Kcal/ 100g
FWT	8.47±0.04^{a,b}	2.39±0.02^{a,b}	6.47±0.18^{c}	3.15±0.56^{b}	5.30±1.44^{a}	74.22±0.36^{a}	351.11±0.68^{a,b}
WTFB-72	9.05±0.08^{a}	2.24±0.07^{a,b}	7.35±0.35^{b}	2.67±0.56^{c}	4.83±0.08^{a}	73.86±0.26^{a}	348.79±0.18^{b,c}
WTF-72	8.60±0.68^{a,b}	2.17±0.11^{b}	8.43±0.39^{a}	2.44±0.04^{c}	4.63±0.54^{a}	73.73±1.42^{a}	350.60±3.8^{a,b,c}
FRT	8.40±0.05^{a,b}	2.45±0.53.^{a,b}	6.36±0.80^{c}	3.63±0.20^{a}	4.94±0.53^{a}	74.22±1.06^{a}	354.99±2.94^{a}
RTFB-72	8.95±0.09^{a}	2.32±0.73^{a}	7.38±0.62^{b}	2.65±0.29^{c}	4.86±0.81^{a}	73.84±0.25^{a}	348.73±4.09^{c}
RTF-72	8.84±0.80^{b}	2.18±0.05^{a,b}	8.75±0.33^{a}	2.49±0.08^{c}	4.82±0.35^{a}	73.92±1.65^{a}	349.09±4.57^{a,b,c}

Values are means of triplicate (±SD), means not sharing a common superscript letter in a column are significantly different at ($p<0.05$) as assessed by Duncun Multiple range tests.

NB: FWT: Flour of White Tef

WTFB 72: White Tef Fermented for 72 hours and Baked

WTF-72: White Tef Fermented for 72 hr.

FRT: Flour of Red Tef

RTFB-72: Red Tef Fermented for 72 hr. and Baked

RTF-72: Red Tef Fermented for 72 hr.

4.1.2 Total Ash

In the current investigation, fermentation and baking process decreased the ash contents of tef (Table5) The values for fermented and baked white tef (Dz-Cr-387) were 2.24% and 2.17% and the values for fermented and baked red tef (Dz-01-99) were 2.32% and 2.18% respectively. The reduction was not significant $p>0.05$(Table 5). The level of ash in food is an important nutritional indicator for mineral density and a quality parameter for contamination (Mosha and Vicent, 2005); these minerals may include Ca, K, P, Fe, Na, Zn and Mg and others at varying amounts. The total ash content of fermented white and red tef was reported to be 2.04% and 2.10%, respectively (Yewelsew *et al.*, 2007).Similar trends were reported by (Experimental Station Bulletin 66, 1969; EHNRI, 1998). The observed decrease in ash content of tef flour samples during fermentation might be due to leaching of minerals during the discarding of liquid in the fermentation broth (Gisbson, 1989).

4.1.3. Crude Protein

The protein content of the two varieties as a result of processing increased significantly ($p<0.05$). The values for the fermented and baked white tef (DZ-Cr-387) were 7.35 % and 8.43 % and the values for fermented and baked red tef (DZ-01-99) were 7.38% and 8.75% respectively (Table 5). In general, the increase in protein content can be attributed to microbial synthesis of protein from metabolic intermediates during the growth cycles. Elyas *et al.*, (2002) and Abdeheleen *et al* (2008) reported that the observed increase in protein content after fermentation was probably due to shift in dry matter content through depletion. The growth and propagation of the microorganism responsible for the fermentation of tef

could have contributed to increment of the protein. Therefore, microorganism used for of fermentation of tef might have results an observable increase in protein content (Ejigu *et al.*, 2005). In most human diets; the protein is more limiting than others. Therefore, application of fermentation process that appears to increase the protein content even at the expense of other nutrients may be advantageous during fermentation as evidenced by decrease in ash content (Table 5) attributed to a net synthesis of enzymatic protein by fermenting seeds (Inyang and Zakari, 2008). When we compare the results of the current study with that of the previous studies different observation have been recorded by Melatu Hailu, 1996). The values for fermented and baked white tef were 6.42% and 6.57% and the values for fermented and baked red tef were 6.02% and 6.12% respectively. The same trends of observation have been recorded by Bayehu (2005) for both varieties of tef cultivars.

4.1.4 Crude Fat

The values for fermented and baked white tef (DZ-Cr-387) were 2.67% and 2.44% and the values for fermented and baked red tef (DZ-01-99) were 2.65% and 2.49% respectively. There was a significant difference ($p<0.05$) among the processing treatments. Maximum reduction was observed during fermentation. This reduction in fat content of tef flour during fermentation might be attributed to the increased activities of the lipolytic enzymes during fermentation, which hydrolyze fats to fatty acids and glycerol. Again for the synthesis of protein the primary energy sources are lipids and carbohydrates. So, far during fermentation the lipids will be utilized as energy source results the total fat content decrease. Accordingly the total lipids content has shown decrease as fermentation proceeds.

Moreover, in the same investigation, it has been reported that after 72 hrs of fermentation lipase inhibitors activity will be decreased (Ejigui *et al.*, 2005)

The decrease fat contents of tef flour during fermentation may help to extend the shelf life of the product. The fermented flour with low values of fat has better shelf life than similar products with high fat content. In contrast, food products containing high fat are susceptible to both hydrolytic and oxidative or enzymatic rancidity and responsible for both the general acceptability and storage stability of the product. The results obtained were not similar with the previous studies undertaken by Camis (1960). The values for fermented and baked white tef were 2.14% and 2.32% and the values for fermented and baked red tef were 2.04% and 2.17%of fermented and baked red tef. Similarly Visco (1965), Borison and Laiyl (1967), Seifu (1989) and Doris (1997) studies shows the same trend on different cultivars of tef.

4.1.5 Crude Fiber

There was no significant difference ($p>0.05$) between the two tef samples varieties in their fiber content (Table5). The values for fermented and baked white tef (DZ-Cr-387) were 4.83% and 4.63% and the values for fermented and baked red tef (DZ-01-99) were 4.86% and 4.82%, respectively. The present study, showed a decrease in crude fiber content, whereas results of previous studies (Experimental Bulletin 66, 1969; Doris, 1997) the values for fermented and baked white tef were 4.12% and 3.98% and the values for fermented and baked red tef were 4.04% and 3.87%. The expected decrease in fiber content during

processing could be attributed to the partial solubilisation of cellulose and hemicellulose type of materials by microbial enzymes. (Inyang and Zakari,2008).

4.1.6. Utilizable Carbohydrates

Utilizable carbohydrate content was determined by difference this means there was no analysis conducted for utilizable carbohydrate determination. Instead it was determined using the formula (i.e. utilizable carbohydrate=100- (fat + fiber + protein + ash + moisture) the multiple comparison test has showed that there was no a significant difference ($p>0.05$) between the treatments of samples in their carbohydrate content (Table 5).

The current study indicated a decreasing pattern during processing. The values for fermented and baked white tef (DZ-Cr-387) were 73.86% and 73.73% and the values for fermented and baked red tef (DZ-01-99) were 73.84% and 72.92% respectively. When we compare results of the current study with the previous findings, it shows different trend with (Melatu Haile, 1996; Doris, 1997 and Bayehu Hailu, 2005). The reason behind these observed reductions could be due to the fact that starch and soluble sugars are principal substances for fermenting microorganisms. Therefore degradation and a subsequent decrease in starch content are expected to occur. Fermentation will activate the enzymes which act on polysaccharides. These enzymes can degrade both high and low molecular weight soluble polysaccharides from cereals. Thus, there will be degradation of polysaccharides components which latter leads to reduction of utilizable carbohydrates.

4.1.7. Gross Energy

The total energy in (Kcal) is calculated mathematically using the equation:-.

Total energy (kcal) =9*fat) + (4*carbohydrate) + (4*protein). Both tef samples varieties have a significant effect on the total energy content ($p< 0.05$).The values for fermented and baked white tef (DZ-Cr-387) were 348.79% and 350.60 % and the values for fermented and baked red tef (DZ-01-99) were 348.73% and 349.09%, respectively (Table 5). The decreasing in energy might be due to increase in alpha amylase activity breaks down complete carbohydrate to simpler and more absorbable sugars which are utilized by the microorganisms during the early stages of fermentation.

4.2. Effect of processing on the mineral contents of tef

4.2.1 Iron

The iron content of the two tef varieties in mg/100g is given in Table 6. The values for fermented and baked white tef (DZ-Cr-387) were 11.10 mg/100g, 11.78 mg/100g and the values for fermented and baked red tef (DZ-01-99) were 11.13 mg/100g and 11.38 mg/100g, respectively. In both fermentation and baking treatments the relation were not significant ($p>0.05$). The reduction of Iron due to fermentation process could arise because of all the water soluble minerals are often lost with the fermentation medium. On the other hand, reduction on the iron content of the samples after fermentation and baking may be described by utilization of some of the hydrolyzed elements by microorganisms for their metabolic activities and lost through decantation. Thus minerals could have been lost in the fermentation medium and decant of fermentation water during the drying process. The iron

content in the present study of tef samples was different from previously reported values of iron 9.01mg/100g and 9.91mg/100g for fermented and baked white tef and 10.55 mg/100g and 10.72mg/100g for fermented and baked red tef (Yewelsew *et al.*, 2007; Experimental Bulletin 66, 1969 and EHNRI, 1998).

Table 6. Effect of Processing on mineral content of tef.

Sample	Iron (mg/100g)	Zinc (mg/100)	Calcium (mg/100g)
FWT	$12.82\pm0.54^{a,b,c}$	3.68 ± 0.43^{b}	$33.78\pm4.76^{a,b}$
WTFB-72	11.10 ± 0.96^{a}	4.10 ± 0.19^{d}	$25.11\pm1.33^{a,c}$
WTF-72	11.78 ± 0.39^{a}	3.76 ± 0.67^{c}	31.49 ± 0.97^{b}
FRT	$12.08\pm0.20^{a,b}$	4.33 ± 0.44^{d}	36.13 ± 1.85^{a}
RTFB-72	$11.13\pm0.97^{a,b,c,}$	4.37 ± 2.39^{c}	$33.67\pm1.55^{a,b}$
RTF-72	$11.38\pm0.21^{a,b}$	4.85 ± 2.32^{a}	35.99 ± 1.21^{a}

Values are means of triplicate (±SD), means not sharing a common superscript letters in a column are significantly different at $p<0.05$ as assessed by Duncan multiple range tests.

4.2.2. Zinc

The process applied in the current study of zinc had shown an increasing trend in the zinc content. The values for fermented and baked white tef (DZ-Cr-387) were 4.10 mg/100g and 3.76 mg/100g and the values for fermented and baked red tef (DZ-01-99) were 4.37 mg/100g and 4.85 mg/100g, respectively. The total zinc content was significantly different $p<0.05$ (Table 6). The possible explanation for the increment in Zinc content in both types of samples might be contamination from the water used for fermentation. This could be attributed to contamination of sample from materials on ashing as well as the lining of the furnace. Dissolved metal ions present in the distilled water used for analysis of samples preparation can also influence the results obtained. However, the finding of the current study was not in agreement with the previous studies (Kelbesa Urga and Narasim, 1998; Melatu Haile, 1996 and Yewelse *et al.*, 2007). The values for fermented and baked white tef were 3.22mg/100g and 3.04mg/100g and the values of fermented and baked red tef were 3.98mg/100g and 3.05mg/100g of fermented and baked red tef.

4.2.3. Calcium

The calcium contents of both varieties of tef are recorded in (Table 6). The values for fermented and baked white tef (DZ-Cr-387) were 25.11 mg/100g and 31.49 mg/100g and the values for the fermented and baked red tef (DZ-01-99) were 33.67 mg/100g and 35.99 mg/100g, respectively (Table 6).

The range of calcium content of the tef samples of the two varieties of the same species was reported with in 127 – 130 mg/100g (EHNRI, 1998) 124 – 154 mg/100g (Yewlsew *et al.*, 2007). The values in the current study was lower than this range and not significant in its relation ($p>0.05$).

The foods prepared from the two tef varieties analyzed were not rich in calcium, consistent with earlier reports (EHNRI, 1998; Melaku *et al.*, 2005). Further, beside the reasons given for other minerals (Iron, Zinc) the relative consistency of the calcium content with in the food type strongly suggests that soil contamination did not contribute to their calcium content (Yewlsew *et al., 2007)*

4.3 Effect of processing on the anti nutritional content of tef

4.3.1. Phytic Acid (phytate)

There was a significant difference ($p<0.05$) between treatments (Table 7).The values for fermented and baked white tef (DZ-Cr-387) were 142.15 mg/100g and 115.27 mg/100g and the values for fermented and baked red tef (DZ-01-99) were 153.16 mg/100g and 146.68 mg/100g, respectively. The highest reduction of phytate was observed in the fermentation and baking processes. Fermentation reduced the phytate 70-90% compared to raw samples. This is in agreement with the previous studies (Melaku et al., 2005; Yewlsew et al., 2007). Which were 155 mg/100g and 121 mg/100g of fermented and baked white tef and 145 mg/100g and 138 mg/100g for fermented and baked red tef.

Elkail *et al.*, (2000) used natural fermentation process and achieved up to 83% reduction in phytate. Reduction in phytic acid contents of cereals with processing treatments has been frequently reported by (Ibrahim *et al.*, 2002). This has been attributed to an increase of phytase activity of the phytate that make the phytate soluble and release bound protein and minerals.

Table 7: Effect of processing on the Anti nutritional content of tef

Factors	Treatments					
	FWT	**WTFB-72**	**WTF-72**	**FRT**	**RTFB-72**	**RTF-72**
Phytate mg/100g	154.50±3.11^{a,b}	142.15±2.5^{c,d}	115.27±3.15^{e}	162.97±5.37^{a}	153.14±6.09$^{b\,c}$	146.68±2.63$^{c,\,d}$
Tannin mg/100g	86.82±0.40^{a}	80.42±2.096^{e}	78.14±4.03$^{b,\,c}$	80.23±2.90^{b}	74.43±2.31^{c}	76.51±3.03$^{b\,c}$

Values are means of triplicate (±SD), means not sharing a common superscript letter in a raw are significantly different at $P<0.05$ as assessed by Duncun Multiple range tests.

4.3.2. Tannin Content

The analysis of the two varieties of tef processing methods shows significant difference $p<0.05$(Table 7). The values for fermented and baked white tef (DZ-Cr-387) were 80.42 mg/100g and 78.14 mg/100g and the values for fermented and baked red tef (DZ-01-99) were 74.43 mg/100g and 76.51 mg/100g, respectively. The tannin content decreased by 80-90%. In the present study, losses of tannin after processing were in agreement with previous studies (Melaku *et al.*, 2005; Yewlsew *et al., 2007*). Which were in the range of 74.49mg/100g and 73.13mg/100g fermented and baked white tef and 72.02mg/100g and 79.40mg/100g fermented and baked red tef.

The reason behind this reduction might be attributed to the enzymatic hydrolysis by polyphenolase of phenolic compounds (Reddy *et al.*, 1985). Moreover, differences in both phytate and tannin contents for the two tef varieties could be due to both genotypic and environmental conditions. Reduction in tannin contents due to fermentation might have been caused by the activity of polyphenol oxidase or tannase of fermenting microflora on tannin. (Fagbeni *et al.*, 2005)

4.4 Effect of processing on the interaction between phytate and mineral of tef

4.4.1 Phytate: Iron molar ratio

The molar ratio of the current study was given in (Table 8). The values for fermented and baked white tef (DZ-Cr-387) were 1.09 mg/100g and 0.83mg/100g and the values for fermented and baked red tef (DZ-01-99) were 1.03 mg/100g and 0.81 mg/100g, respectively. In previous studies the values were in the range of 0.4 to 1.9 and 0.1 to 1.3 for both samples (Yewlsew *et al., 2007*).

Phytate: iron ratios > 0.15 is an indicator for low iron bioavailability (Melaku *et al.*, 2005). All samples analyzed contain phytate: iron molar ratios of > 0.15. However fermentation of tef grain resulted in reduction of the phytate: iron molar ratios .In general, there might be an increase in mineral content and decrease in phytate content during fermentation.

Table 8 Effect of processing on phytate: Iron, phytate: Zinc, phytate: calcium, and (calcium) (phytate)/Zinc content of tef

Sample code	Phytate: Iron * Molar ratio	Phytat:ZincΨ molar ratio	Phytate: calcium β molar ratio	(phytate)X(calcium) / zinc ¥ (mol/kg)
FWT	0.82±0.13[b]	3.27±0.38[c]	0.28±0.002[b]	2.95±0.38[c d]
WTFB-72	1.09±0.75[a]	6.69±0.77[a]	0.34±0.012[a]	4.20±0.69[b]
WTF-72	0.83±0.05[b]	3.18±0.68[c]	0.22±0.001[d]	2.51±0.61[d]
FRT	1.15±0.07[a]	6.89±0.94[a]	0.27±0.005[b]	6.33±0.59[a]
RTFB-72	1.03±0.79[a, b]	4.48±0.13[b]	0.27±0.002[b]	3.76±0.06[b, c]
RTF-72	0.81±0.07[b]	2.70±0.17[c]	0.25±0.004[c]	2.43±0.23[d]

Values are Means of triplicate (±SD), means not sharing a common superscript letters in a column are significantly different at $p<0.15$ as assessed by Duncan multiple range tests.

*= (mg of phytate/MW (Molecular weight) of phytate: mg of Iron/MW of iron

Ψ= (mg of phytate/MW (Molecular weight) of phytate: mg of Zinc/MW of Zinc

β= (mg of phytate/MW (Molecular weight) of phytate: mg of Calcium/MW of calcium

¥= (mol/Kg calcium) X (ml/Kg phytate)/ (mole/kg Zinc)

4.4.2. Phytate: Zinc molar ratio

The phytate: Zinc molar ratio values for fermented and baked white tef (DZ-Cr-387) were 6.69±0.77 mg/100g and 3.18±0.68 mg/100g the values for fermented and baked red tef (DZ-01-99) were 4.48±0.13mg/100g and 2.70±0.17mg/100g respectively .Table 8 and a few previous studies indicate that the phytate: Zinc molar ratios were in the range 6.6 to 9.2 for white tef and 9.1 to 6.6 for red tef (Yewelsew et al., 2007). In general phytate: Zinc molar ratio is used to estimate the likely absorption of zinc from a diet. Diets with a phytate: zinc molar ratio greater than 15 have relatively low Zinc bioavailability, those with phytate: Zinc molar ratios between 5 and 15 have medium zinc bio availability and those with a phytate: zinc molar ratio less than 5 have relatively good zinc bioavailability (Adeyeye *et al.*, 2000; Melaku *et al.*, 2005; Khakoni M, 2009).

In the present study low values (phytate: Zinc molar ration < 15) were found in samples fermented for 72 hrs. And gives phytate: Zinc molar ratios of less than 10.According to Melaku *et al.*, 2005. Children in Ethiopia are especially very prone to deficiencies of mineral and trace elements, as they eat from family dish and often cannot meet their specific nutrients needs, Thus supplementation with Zinc increases the linear growth of infants, particularly those who were stunted. Hence, phytate: Zinc molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone. (Kelbesa and Narhsimhe, 1998; Adeyeye *et al.*, 2000). The decrease in the phytate: Zinc molar ratio during fermentation on both varieties realized by bioavailability of Zinc could be attributed to ability of the fermentation microorganisms to hydrolyze more phytate due

to the reduction of PH that favors phytate activity without the relative interference of tannins in absorption of minerals.

4.4.3 Phytate: Calcium ratio

There was a significance difference between the fermented and baked samples of both tef varieties ($p< 0.05$).The values for fermented and baked white tef (DZ-Cr-387) were 0.34±0.012mg/100g and 0.22±0.001mg/100g and the values for fermented and baked red tef (DZ-01-99) were 0.27±0.002 mg/100g and 0.25±0. 004 mg/100g respectively. Table 8. The high calcium content of food may jeopardize bioavailability of iron and zinc. High calcium levels in foods can promote the phytate – induced decrease in zinc bioavailability when the [cacium] x [phytate] / [zinc] molar ratios exceed 0.5 (Melaku *et al.*, 2005). However, values observed in the flour samples indicate the possible contribution of calcium in exacerbating the low bioavailability of zinc and iron due to phytase is probably minimal.

The critical phytate: calcium molar ratio is 1:6. The phytate: calcium molar ration of > 6 is indicative of favorable for calcium absorption. Both the tef flour sample varieties analyzed in this study exhibited phytate: calcium molar ratios less than 6, which indicate that calcium is available for absorption from such diets. The results obtained in this investigation indicate the positive impact of calcium on mineral (iron and zinc) absorption. This is indicative of calcium availability for absorption (Melaku et al., 2005). In general, phytate: mineral ratio was decreased significantly after fermentation for all the parameters examined. Even if the critical values were not achieved in most samples, the

lower phytate: mineral ratios from the fermented two tef varieties may be partly ascribed to the decreased content of phytic acid during fermentation which had a significant correlation ($p< 0.05$) with the phytate: mineral ratio (bioavailability of minerals). Thus fermentation enhances bioavailability of minerals by degrading phytate with microbial and native phatases that entangle macro – and trace – elements, (Kelebesa and Narasimla, 1998; Odumodu, 2007). These studies indicated that fermentation hydrolyzed anti nutrients from their organic bonds to increase mineral bioavailability.

4.5. Effect of processing on phytate phosphorous and non-phytate phosphorous content of tef

There was a decreasing pattern for phytate phosphorous treatments (Table 9) and they are significantly related ($p<0.05$); Miyazawa (1996) concluded that, in various seeds, phytic acid positively correlates with total phosphorous. Factors that affect the total phosphorous such as soil,available phosphorous,varity,climate condition and fertilizer,can influence phytic acid concentration.

Table 9 Effect of processing on total phosphorous, phytate phosphorous and non-phytate phosphorous content of Tef

Sample code	Total phosphorous (mg/100g)	Phytate phosphorous *		Non Phytate phospherouse**	
		Total (mg/100g)	% of total phosphorous	Total (mg/100g)	% of total phosphorous
FWT	$51.27\pm0.85^{d,e}$	$43.49\pm0.88^{b,}$	84.85 ± 3.13^{a}	7.78 ± 1.73^{b}	15.14 ± 3.13^{b}
WTFB-72	48.85 ± 1.53^{e}	40.05 ± 0.74^{d}	82.08 ± 4.03^{a}	8.79 ± 2.24^{b}	17.91 ± 4.03^{b}
WTF-72	66.37 ± 2.16^{b}	32.48 ± 0.89^{e}	49.00 ± 2.92^{b}	33.89 ± 3.04^{a}	50.99 ± 2.92^{a}
FRT	51.18 ± 2.04^{c}	45.92 ± 1.51^{a}	79.07 ± 5.4^{a}	12.25 ± 5.4^{a}	20.92 ± 5.4^{b}
RTFB-72	$53.04\pm1.63^{d,e}$	43.15 ± 1.71^{b}	81.47 ± 5.74^{a}	9.88 ± 3.35^{b}	18.60 ± 5.87^{a}
RTF-72	75.52 ± 2.38^{a}	$41.33\pm0.74^{a,d}$	54.79 ± 2.71^{b}	34.18 ± 3.13^{a}	45.20 ± 2.71^{a}

Values are Means of triplicate (±SD), means not sharing a common superscripts letters in a column are significantly different at $p<0.15$ as assessed by Dun can multiple range tests. *phytate phosphorous content was calculated by assuming that phytate contains 28.18%phosphorous. **non-phytate phosphorous was the difference between total phosphorous.

Fermentation lowered the levels of phytate phosphorous in all the samples with a simultaneous increase in non-phytate phosphorous ($p<0.05$) Table 9. Within 72h of fermentation the non-phytate phosphorous increased by 50.9% and 45.20%for both tef varieties respectively. Thus the hydrolytic reduction of phytic acid during fermentation may have contributed to the bioavailability of phosphorous. Hence, the lower the phytate phosphorous, the more bioavailability of phosphorous in the fermented samples is observed. Generally, diets with phosphorous as phytate (%) $\leq$ 60% are regarded as being adequate in bioavailability of phosphate (Melaku *et al.*, 2005).

The reduction in phytate phosphorous during fermentations may be due to the phytate hydrolysis by phytase elaborated by the fermenting micro flora (Andlid and Sandberg 2002; Hurrel and Reddy, 2003). Cleavage of phosphorous from phytic acid may explain the improved availability of phosphorous in fermented tef varieties. A corresponding decrease in phytate phosphorous and enhancement in the non-phytate phosphorous were noticed in the present study. Therefore consumption of fermented cereals may help to ameliorate prevalent mineral deficiencies caused by their limited bioavailability from the unfermented ones and may lead to better mineral status.

5. Conclusion and Recommendation

5.1 Conclusion

In this nutritional and anti nutritional factors study, six samples were analyzed for five parameters. The two new varieties of Tef [*Eragrotis tef* (zucc.) trotter], White tef (DZ-Cr-387, Qunicho) and Red tef (DZ-01-99) grown in Ethiopia were taken and subjected to three main treatments; flour, baking and fermentation. The parameters analyzed were proximate composition, mineral composition, anti-nutritional factors, molar ratios of phytate and minerals, total phosphorus, phytate-phosphorous and non-phytate phosphorous.

In all the treatments total protein and moisture content have shown increasing values. The rest have shown a decreasing pattern of proximate analysis. The results presented in this study revealed that tef was rich in mineral content especially, Fe, Ca, Zn and P. The values obtained for Fe, Ca and Zn in this study were less than the required daily amount (RDA), but could be augmented by either increasing the quantity of tef consumption or complementing it with other food resources.

To improve the nutrients intake, food preparation technologies have been advocated that will effectively increase the nutrient availability of cereal diets. Therefore technologies must, however be simple, easily understood and culturally acceptable, and the food products must be affordable in terms of economy and labour input.

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

The molar ratios of phytate and minerals also act as an indicator for the bioavailability of minerals in food samples after fermentation and baking. The values of total phosphorous, phytate phosphorous and non- phytate phosphorous showed trend of increment in phosphorous bioavailability. Beside all this, currently the demand for minimally processed foods has increased because of the enhancement of some nutritional and chemical composition of those treatments. Moreover, cost wise the traditional ways of processing foods are advisable due to their lower energy and power consumption. Then it can be concluded from the present study is that antinutritional factors in tef can be degraded by baking and fermentation.

5.2 Recommendations

Now a day there is a great deal of interest in developments of nutritionally rich food sources to meet the demand of population in developing country. In these countries scarcity of balanced diet has lead to sever malnourishment. The possible remedy for these nations malnourishment problem is production of adequate foods having the basic food nutrients. It is therefore safe to recommend that tef does not manifest any special capability for higher nutritional uptake than other cereals.

Even though tef grown in Ethiopia in the pre-Semitic period, no adequate scientific researches worked especially in the nutritional component of tef. Thus the current study with its own limitation has investigated the effect of processing on the nutritional and anti nutritional composition of tef (*Eragrotis tef)*. But the following issues should also be considered as a recommendation in the future works based on the out comes of the current study.

- Further Nutritional studies should be conducted for other varieties of tef.

- In the process of finding out the new varieties of tef not only the breeding system, ecology and agronomy are described but also the nutritional value of the new variety should be announced.

- Agricultural researchers, Food scientists, post harvest technologist etc .should work together in identifying and finding of new varieties of tef.

- The nutritional values of the current varieties should be described in comparison to the previous one.

- The agricultural institute, food science and nutrition institute etc. should state a clear food composition table (nutritional component) of each variety and a clear difference should be indicated in terms of information in all aspect as why it is stated as new.

- Food scientist should give information to the community in the forms of publishing materials about the advantage of Fermentation, baking and ant nutritional factors to the food consumers.

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Declaration

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

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