

Addis Ababa University
School of Graduate Studies
College of Natural Sciences
Food Science and Nutrition program



Uses of Prefermented Flour for Injera Preparation

BY

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A thesis submitted 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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**ADDIS ABABA UNIVERSITY
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The image shows four horizontal lines with handwritten signatures in blue ink. The top signature is a large, stylized cursive signature. The second signature is a dense, scribbled signature. The third signature is a cursive signature with the letters 'IB' clearly visible. The fourth signature is a cursive signature.

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Dedication

This work is dedicated to:

My Husband Birhanu Abera

My Grand Mom W/ro Habesha Melaku

And

My Mother Askale Hunegnawu(Aqua).....You were the best mother in the world before you go to your father(God). I wish you could see this.

Table of Contents

Acknowledgments.....	i
Dedication.....	ii
Table of Contents.....	iii
List of Tables.....	vi
List of Figures.....	vii
List of Abbreviations.....	viii
Abstract.....	x
1. Introduction.....	1
1.1 Background of the study.....	1
1.2 Statement of the problem.....	2
1.3 Objectives.....	3
1.3.1 General objective.....	3
1.3.2 Specific objectives.....	3
2 Literature review.....	4
2.1 Fermentation of Cereals.....	4
2.2 Nutritional benefits of cereal based foods.....	5
2.2.1 Nutritional benefits of cereals in Ethiopia.....	5
2.3 <i>Teff</i> [<i>Eragrotis tef</i> (Zucc.) Trotter].....	6
2.3.1 Proximate composition of <i>Teff</i>	8
2.3.2 Mineral content of <i>teff</i>	9
2.3.3 Antinutritional factors commonly found in cereals.....	10
2.4 <i>Injera</i>	12
2.5 Traditional <i>injera</i> processing.....	12
2.6 Prefermented flour.....	15
2.6.1 Prefermented flour preparation steps.....	15
2.6.2 Use of the prefermented flour.....	16
2.6.3 Advantages of prefermented frozen dough and prefermented flour.....	16
2.7 Different drying methods used for food products.....	17
2.7.1 Sun drying.....	18
2.7.2. Freeze drying (Lyophilization).....	19
2.7.3 Oven drying.....	20

2.7.4 Spray drying.....	20
2.8 Leavening agents	21
2.8.1 Microbial leavening.....	21
2.8.2 Chemical leavening	21
3. Materials and method	25
3.1. Study site	25
3.2. Description of the Variety of Teff Used for Analysis	25
3.3. Procurement of materials	25
3.4. Sample preparation procedures	25
3.3.1 Preparation of prefermented flour	26
3.4 Sensory evaluation.....	30
3.5 Proximate composition determination	30
3.5.1 Determination of moisture content.....	31
3.5.2 Determination of crude protein	31
3.5.3 Determination of ash	32
3.5.4 Determination of crude fat	32
3.5.5 Determination of crude fiber.....	33
3.5.6 Determination of crude carbohydrate.....	34
3.5.7 Determination of gross energy	34
3.6 Determination of antinutritional factors.....	34
3.6.1 Determination of phytic acid	34
3.6.2 Determination of tannins	34
3.7 Determination of minerals.....	35
3.8. Phytate and non phytate phosphorous analysis	36
3.9 Statistical analysis.....	36
4. Results and discussion.....	37
4.1. Prefermented (instant) flour preparation.....	37
4.2. <i>Injera</i> preparation from prefermented flour (optimization trials)	37
4.3 Effect of processing on sensory acceptability of <i>injera</i>	41
4.3.1 Fluffiness	41
4.3.2 Taste.....	42
4.3.3 Eye size and distribution.....	43

4.3.4 Color	43
4.3.5 Appearance.....	43
4.3.6 Aroma	44
4.3.7 Over all acceptability.....	44
4.4 Proximate composition results of injera processing with different driers	45
4.4.1 Moisture content.....	45
4.4.2 Crude protein	46
4.4.3 Total Ash	47
4.4.4 Crude fat content	47
4.4.5 Crude fiber content.....	48
4.4.6 Crude Carbohydrate	48
4.4.7 Gross energy	48
4.5 Effect of processing on antinutritional factors	49
4.5.1 Phytic acid (phytate).....	49
4.5.2 Tannin content.....	50
4.6 Effect of processing on mineral content	51
4.6.1 Iron	51
4.6.2 Zinc.....	52
4.6.3 Calcium.....	52
4.6.4. Phosphorous.....	53
4.7. Phytate and non phytate phosphorus content	53
4.7.1 Phytate phosphorus content	53
4.7.2. Non phytate phosphorus content	54
5 Conclusion and recommendation.....	55
5.1 Conclusion.....	55
5.2 Recommendations.....	56
6 References	57
7 Appendixes.....	67

List of Tables

Table 1 Importance of cereals in diet of Ethiopia (Rashid, 2010)	6
Table 2 Chemical composition of <i>teff</i> grain (Bultosa, 2004; Umeta, 2006).....	9
Table 3 Sensory characteristics of <i>teff injera</i> prepared from prefermented flour prepared with different drying methods	42
Table 4 Proximate composition tef injera prepared from prefermented flour prepared with different drying methods.	46
Table 5 Phytate and condensed Tannin composition of <i>teff injera</i> prepared from prefermented flour prepared with different drying methods.....	50
Table 6 Mineral content of <i>teff injera</i> prepared from prefermented flour prepared with different drying methods	51
Table 7 Phytate and non phytate phosphorous composition of <i>teff injera</i> prepared from prefermented flour prepared with different drying methods	54

List of Figures

Figure 1 Flow diagram for the preparation of injera.....	14
Figure 2 Flow diagram of process followed in injera preparation from prefermented instant flour	27
Figure 3 Injera preparation from prefermented flour with addition of cold water	27
Figure 4 <i>Injera</i> preparation from prefermented flour with addition of hot (43 °C) water.....	29
Figure 5 <i>Injera</i> preparation from prefermented flour with addition of back sloped starter	29
Figure 6 Injera preparation from prefermented flour with addition of "Absit"	29
Figure 7 Injera preparation from prefermented flour with addition of baking powder	30
Figure 8 <i>Injera</i> prepared from prefermented flour mixed with cold water	38
Figure 9 Injera prepared from prefermented flour mixed with hot water (43°C).....	39
Figure 10 <i>Injera</i> prepared from prefermented flour mixed with water and 'absit'	39
Figure 11 <i>Injera</i> prepared from prefermented flour mixed with water and back sloped starter culture.....	40
Figure 12 <i>Injera</i> prepared from prefermented flour, water and baking powder (10min.).	40

List of Abbreviations

AACC	Approved methods of American Association of Cereals Chemists
AOAC	Association of official chemists
ANOVA	Analysis of variance
a_w	Water Activity
°C	Degree Centigrade
Ca	Calcium
CHO	Carbohydrate
CSA	Central Statistical Agency (of Ethiopia)
DZARC	Debreziet Agricultural Research center
EHNRI	Ethiopian Health and Nutrition Research Institute
g	Gram
h	Hour
HCl	Hydrochloric Acid
HNO ₃	Nitric Acid
H ₂ SO ₄	Sulphuric Acid
H ₂ O	water
H ₂ O ₂	Hydrogen Peroxide
Fe	Iron
Kg	Kilogram
l	Litre
min	Minute
mg	Milligram
ml	Millilitre

mm	millimeter
NaOH	Sodium Hydro oxide
P	Phosphorous
Phy	Phytate
ppm	Parts Per Million
SD	Standard Deviation
SPSS	Statistical Product and Service Solutions
USA	United States of America
Zn	Zinc
%	Percent

Abstract

The study attempted to investigate the possibility of using prefermented instant flour for the production of injera by using different drying methods. The effects of drying methods used were compared with the traditional method in chemical composition and sensory properties of the products. Optimization of using prefermented flour had also been done. The result in sensory analysis gave fluffiness score of 6.30, 6.40 and 7.74 for oven dried; sun dried and freeze dried samples respectively. Taste was scored as 5.24, 6.18, and 6.94 for sun dried, oven dried and freeze dried respectively. Eye size and distribution was scored as 5.44, 6.72 and 7.56 for oven dried, sun dried and freeze dried respectively. Color was scored as 6.22, 6.30 and 7.76 for sun dried; oven dried, and freeze dried samples respectively. Appearance was scored as 6.16, 6.16, and 7.96 for sun dried; oven dried, and freeze dried samples respectively. Aroma was scored as 5.18, 5.84, and 7.18 for oven dried, sun dried and freeze dried respectively. Overall acceptability was scored as 5.74, 6.22 and 7.68 for sun dried, oven dried and freeze dried respectively. The nutritional composition gave a value of moisture content 53.57, 53.83 and 56.4 g/100g for oven dried, sun dried and freeze dried respectively. Crude protein content was 8.73, 9.05 and 10.03 for sun dried, freeze dried and oven dried respectively. Ash content was 4.65, 3.53 and 4.65 (g/100g) for sun dried, oven dried and freeze dried respectively. Crude fat content was 2.75, 2.62 and 3.52 g/100g for freeze dried sun dried and oven dried respectively. Crude fiber content was 1.91, 2.23 and 2.67g/100g for oven dried, sun dried and freeze dried respectively. The iron content was 12.12, 13.27, and 14.18 g/100g for freeze dried; oven dried and sun dried samples respectively. Zinc content was 1.51, 1.60 and 1.63(g/100g) for freeze dried, oven dried and sun dried respectively. Calcium content was 33.62, 33.65 and 34.95 for freeze dried samples respectively. Phosphorous content was 507.85, 567.85 and 659.80(g/100g) for freeze dried, sun dried and oven dried respectively. Phytate content was 232.55, 248.9 and 264.97 for oven dried sun dried and freeze dried samples respectively. Tannin content was 35.43, 36.58, 38.29mg/g for oven dried, sun dried and freeze dried samples respectively. Phytate phosphorus content was 63.47, 79.92 and 81.46mg/100g for oven dried sun dried and freeze dried samples respectively, while non phytate phosphorus content was 432.93, 499.15 and 596.33mg/100g for freeze dried, sun dried and oven dried samples respectively.

Key words: *Prefermented flour, freeze drying, oven drying, sun drying, injera*

1. Introduction

1.1 Background of the study

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

Achi (2005) stated that traditional fermented foods are usually made under primitive conditions, which result in low yield and inconsistent quality. In addition to this, preparation of traditional fermented foods is time consuming. According to Nout (1992) the art of traditional processes needs to be transformed into a technology to standardize quality of the end products without losing their desirable traits.

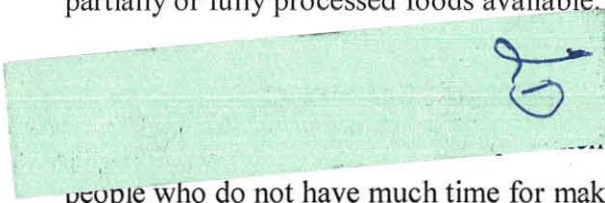
Several innovations have been made to solve different problems of bakery products. For instance, pre-fermented frozen dough has been used to bake fermented products by the home users with short period of time by purchasing pre-fermented frozen dough (Jane, 1998). Another innovation was the use of pre-fermented flour in bakery industries. In this case, the flour is made after the fermented dough is dried and grounded into flour. The pre-fermented flour can then be used to make bakery products with no further fermentation by the user (Hoffman, 1976). The use of chemical leavening agents was also another development in the bakery industry (Bellido, 2009). Chemical leavening systems can leaven bread dough faster, more consistently and conveniently than yeast because they do not require lengthy activation periods or strict environmental conditions for evolving gas. The chemical nature of chemical leavening systems makes them capable, once incorporated into the dough, of producing carbon dioxide in a typical processing or storage conditions such as at high extrusion temperatures, in microwave fields, and prolonged refrigeration or frozen storage (Bellido, 2009).

Although *injera* is a staple food for majority of Ethiopians, its preparation takes several days because it involves longer fermentation process. According to Hamaker, *et al.* (2007), as industrialization expand and civilization achieved, people will be busy and face time constraints to cook foods with longer process. Due to these reasons they prefer to use processed or semi processed foods from industries. These interests are started to be reflected in urban societies of Ethiopia. Some people are buying baked *injera* from shops rather than baking at home.

The present study aims to provide a method for producing prefermented flour. The prefermented flour is easy to convert it to *injera* and saves time for the home user. The study further investigates optimization of prefermented flour and evaluate its effect on the sensory quality and chemical composition of *injera*.

1.2 Statement of the problem

As industrialization expand and countries get civilized, people will be busy and face time constraints to cook foods with longer process and prefer to use processed or semi processed foods from industries (Hamaker, 2007). About 85% of the population in Ethiopia is engaged in agricultural activities as the main source of employment. But recent developments have shown that the prospects for employment generation in other sectors are promising and many new jobs have been created (Ethiopia, 2009). This transformation into industrial based economy needs transformation in traditional food processing practices. Therefore, there is a need to have partially or fully processed foods available.

 and also getting popularity in developed countries its lack of some basic skills is discouraging to be used by people who do not have much time for making it at home. In addition to this, Ethiopians living in other countries always complain that they do not usually get the real taste of *injera* which seems that there are some environmental factors which affect some fermentation characteristics of *injera*. There is therefore, a need to optimize its preparation so that it can be easily accessible by consumers who don't have time and/or skill for *injera* preparation as well as to get the real taste wherever it is prepared. There is no any other prior research on this area other than (Hoffman, 1976) who takes the patent to prepare the prefermented flour for fermented sour bread product.

1.3 Objectives

1.3.1 General objective

The overall objective of this study was to assess the uses of prefermented flour produced by different drying methods for *injera* preparation and evaluate the chemical composition and sensory acceptability of the final product.

1.3.2 Specific objectives

The following were the specific objectives of this study:

- Prepare prefermented flour for *injera* preparation using different drying methods.
- Optimize the steps of *injera* preparation using prefermented flour prepared in different drying methods.
- Evaluate the sensory quality of *injera* prepared from prefermented flour dried by different drying methods.
- Evaluate the nutritional and anti nutritional content of *injera* prepared from prefermented flour dried by different drying methods.

2 Literature review

2.1 Fermentation of Cereals

Fermented foods are those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food (Beuchat, 1983). In other words foods which are submitted to the influence of lactic acid producing microorganisms are considered as fermented food (Fellows, 2000). Traditional fermentation is a form of food processing, where microbes for example, lactic acid bacteria are utilized. The bacteria used food as a substrate for their propagation. This is a form of food preservation technology, used from ancient times. Over the years, it became part of the cultural and traditional norm among the indigenous communities in most developing countries, especially in Africa. This has made fermented foods one of the main dietary components of the developing world (Zewdu, 2008). Cereal grains had been one of man's earliest sources of food. One way of processing the grains into food is through fermentation (Osungbaro, 2009).

The changes occurring during the fermentation process are mainly due to enzymatic activity exerted by the microorganisms and/or the indigenous enzymes in the grain. This bioprocess causes considerable changes that will affect the organoleptic properties (taste and viscosity), the nutritional value and the microbial safety of the food (Lorri, 1997). Fermentation makes the food palatable by enhancing its aroma and flavor. These organoleptic properties make fermented food more popular than the unfermented one in terms of consumer acceptance (Vilas, 2010).

A number of foods especially cereals are poor in nutritional value, and they constitute the main staple diet of the low income populations. However, fermentation has been shown to improve the nutritional value and digestibility of these foods. The acidic nature of the fermentation products enhances the activity of microbial enzymes at a temperature range of 22-25 °C (Vilas, 2010). The enzymes, which include amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively. Thus, in addition to enhancing the activity of enzymes, fermentation also reduces the levels of antinutrients such as phytic acid and tannins in food leading to increased bioavailability of minerals such as iron, protein and simple sugars. The preservative activity of fermentation has been observed in some fermented products such as cereals. The lowering of pH to below 4

through acid production inhibits the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease (Poutanen, 2009).

2.2 Nutritional benefits of cereal based foods

The nearly ubiquitous consumption of cereals all over the world gives cereals an important position in international nutrition (Dewettinck, 2008). Besides the high starch content as energy source, cereals provide dietary fiber and nutritious protein lipids. One way of processing the grains into food is through fermentation. Fermented cereals are very widely utilized as food in African countries and in fact cereals account for as much as 77% of total caloric consumption. A majority of traditional cereal based foods consumed in Africa are processed by natural fermentation and are particularly important as weaning foods for infants and as dietary staples for adults. These fermented cereal based food products can be classified on the basis of either the raw cereal ingredients used in their preparation or the texture of the fermented products. The major cereal based foods are derived mainly from maize, sorghum, millet, rice, or wheat. Pre-fermentation treatments of cereals are largely dependent on the type of cereal and on the end products desired. Generally, treatments such as drying, washing, steeping, milling, and sieving are some of the processing steps applied in the preparation of these fermented cereal foods (Osungbaro, 2009).

2.2.1 Nutritional benefits of cereals in Ethiopia

In terms of caloric intake, cereals dominate the diets of Ethiopian households (Table 2.1). It is suggested that an average Ethiopian consumes foods that produce 1858 kilocalories. Of the total calorie consumption, four major cereals (maize, *teff*, wheat, and sorghum) account for more than 60 percent, with maize and wheat representing 20 percent each. The low share of *teff* in calorie consumption often come as surprise to urban Ethiopians, as *teff* is the predominant staple in the of the middle- and high- income households (Rashid, 2010).

Table 1 Importance of cereals in diet of Ethiopia

Commodities	Daily caloric intake (cal./Day)	Percentage of daily caloric intake (%)
Maize	383	20.6
Wheat	364	19.6
Teff	254	13.7
Sorghum	191	10.3
Other	666	35.8
Total	1858	100

Source: (Rashid, 2010)

2.3 Teff [*Eragrostis tef* (Zucc.) Trotter]

Teff (*Eragrostis tef* (Zucc.) Trotter) is a major cereal crop cultivated in Ethiopia. It occupies approximately two million hectares of land each year (Dagne, 2008). *Teff* is well known by Ethiopians for its superior nutritional quality. It provides about two-thirds of the daily dietary protein intake of most Ethiopians (Mengesh, 1966). *Injera* is a staple food in Ethiopia (Bultosa, 2007). The whole grain is ground into flour that can be used as a base ingredient for leavened flatbreads such as *injera*, added as a thickening agent to soups and sauces, fermented to make beer and ethnic beverages, or made into porridge and puddings (Adebowale, 2011).

There is a growing interest on *teff* grain utilizations because of nutritional merits (whole grain), the protein is essentially free of gluten. The type of protein found in wheat and it makes alternative food for consumers allergic to wheat glutens. The grain proteins are also presumed easily digestible because prolamins are very small. *Teff* grain micronutrient is also apparently high, particularly in iron, as a result of contamination from soil and also due to fermentation which increases bioavailability of iron in *injera* agronomic practices used in Ethiopia and

fermentation on *injera* making. Because of this, the prevalence of iron deficient anemia among *teff injera* consumers in Ethiopia is low (Bultosa, 2007).

Teff is endemic to Ethiopia and its major diversity is found only in that country as with several other crops, the exact date and location for the domestication of *teff* is unknown. However, there is no doubt that it is a very ancient crop in Ethiopia, where domestication took place before the birth of Christ. *Teff* was introduced to Ethiopia before the ancient introduction of barley. The current area of cultivation is probably not the initial one of domestication; domestication probably occurred in the western area of Ethiopia, where agriculture is precarious and seminomadic (Refera, 2001). According to Refera(2001) *teff* grain commands premium price among other cereals cultivated in Ethiopia.

Recently the use of *teff* in food systems is gaining popularity as both a naturally gluten-free alternative to wheat products and a nutrient-rich ingredient in the baby food industry. However, despite the growing interest in *teff*, there is limited scientific knowledge on some of its characteristics. *Teff* flour, despite it being gluten-free, has been reported to produce high-quality leavened flatbread that stales much slower than if made from other cereals, in particular sorghum (Adebowale, 2011), countries like USA, Canada, Australia, South Africa, and Kenya began production of *teff* for different purposes such as forage crop, thickener for soups, stews and gravies probably because *teff* flour paste gives the product a short stiff texture and *teff* flour imparts a slight molasses-like sweetness to food products, making the inclusion in porridges, pancakes, biscuits (Solomon, 2007; Zewdu, 2008).

In the preparation of *teff* flour the grain will be cleaned by manual sifting. The cleaned grain is usually dry-milled to obtain whole flour. Traditionally in Ethiopia, this was done by *Wofcho* (top and bottom hard stones). Today, milling with hand, using hard stone, has been replaced by grist mills run by electric power, and, where electric power is not available, by diesel engine or water power. The grist mill is made up of two abrasive hard-disk stones. During operation, one stone is stationary while the other is moving back and forth. The grain, fed into the center (eye) of the upper stone, is fragmented and ground between the two stones, and flour is released at the periphery.

Teff is considered to have a better food value than the major grains; namely, wheat, barley, and maize, as it is normally used as a whole grain, i.e., the germ and bran are consumed along with

the endosperm. *Teff* flour is used primarily for making of *injera* (Zegeye, 1997). The flour is also used to make sweet unleavened bread called *kitta*. *Kitta* can be consumed as bread or it can be used as an adjunct in traditional opaque beer (*tella*). Porridge (*genefo*) can also be made from *teff* flour. Thin, fermented *teff* flour batter is used to prepare soup (*muk*). Unfermented *teff* flour dough is also used in the preparation of traditional snacks (*dabbo Kolo*), where the dough is rolled into small balls and then roasted on a hot griddle. Processing of *teff* grain in Ethiopia has been limited to the household level. upto date, technologies for large-scale commercial processing of *teff* grain, for the preparation of foods like *injera* is not well advanced (Taylor, 2004; Bultosaa, 2002)

2.3.1 Proximate composition of *Teff*

The proximate composition of *teff* has been reported with different authors differently. A summarized data is presented in table 2.2. *Teff* grain is reported to contain 9-11% protein, an amount slightly greater than in normal sorghum, maize, or oats. However, samples tasted in the United States have consistently shown even higher protein levels: 14-15% (American Research, 1996). According to the report of Ashenafi *et al.* (2006) total protein content of different cultivars of *teff* ranged from 6.5- 9.3%. Another report also showed that grain protein of different varieties of *teff* ranged 8.7-11.1%. The crude fat had ranged 3.0-2.0% as reported by (Bultosa, 2007). The crude fiber had ranged 2.6-3.8 % with mean 3.3%. Apparently the crude fiber observed in 13 varieties of *teff* is almost similar with a report of 2.0-3.5% with typical value 3.0%. The crude fiber was highest in brown *teff* varieties. *Teff* is consumed as a whole grain, bran and cell wall materials were reported not affected as such on *teff* fermentation on *injera* making. The crude fiber comprises materials that had resisted digestions. These are presumably major contributor for the dietary fiber of characteristic large stools bulk on *teff injera* consumption (Bultosa, 2007). The ash content had ranged 1.99-3.16% with mean of 2.45% as reported by review report of (Taylor, 2004). The ash level in *teff* grain had ranged 2.66-3.00% with typical value 2.8%. Apart from the genetics, the ash levels in *teff* grain are influenced by the agronomic practices used (i.e., by the degree of *teff* grain unseen surface contamination mostly from the threshing floor (Bultosa, 2007). Carbohydrate content of *teff* grain is 73%, of which virtually all is starch (Taylor, 2004).

Table 2 Chemical composition of *teff* grain

Biochemical class	Range	Typical value
Protein%	9.38-13.3	11
Carbohydrate%	73	73
Crude fiber%	1.98-3.5	3
Fat%	2.00-3.1	2.5
Ash%	2.66-3.00	2.8
Food energy kJ/100g	1406	1406
Phytate mg/100g	707	389
Tannin mg/100g (teff injera)	54.8–64.7	60.1

Source: (Taylor , 2004; Umeta, 2006)

2.3.2 Mineral content of *teff*

Teff contains more calcium, copper, zinc, aluminum (Noori, 2010) and barium than winter wheat, barley and sorghum (Ketema, 1997). The total iron content of *teff* is reported to be 0.0033% and 0.0036 - 0.0078mg/100g. Fermentation increased the bioavailable portions of iron from 9% to 24%, phosphorus from 16% to 60% and zinc from 2% to 43%. The increase in dialyzable iron might have a positive effect on its bioavailability, and might thus explain the rarity of iron-deficiency anemia among *teff* consuming population of Ethiopia. (Ashenafi, 2006).

The Fermentation of *injera* reduced the zinc concentration. The iron content of *teff injera*, both fermented and unfermented was reported very high (430 mg/100 g) and about 3× that of sorghum *injera* and about 8× that of *injera* made from wheat or maize (3–4 mg/ 100 g). *Teff*

injera was also rich in calcium, containing 3x that of maize and wheat *injera* and 5x that of sorghum *injera*. The differences in phosphorus content among the *injera* prepared from the different grains were less pronounced. Fermentation reduced the phytate content of *injera* prepared from *teff* and sorghum by a factor of 3–4x and this effect is also seen in the zinc: phytate molar ratio, calcium: phytate molar ratio and the proportion of phosphorus as phytate (Umeta, 2006).

2.3.3 Antinutritional factors commonly found in cereals

Cereal foods are important components of the daily diet, providing carbohydrates, proteins, dietary fibers and vitamins. Epidemiological studies have indicated protective role of whole grain foods against several diseases associated with westernized societies such as type 2 diabetes cardiovascular diseases and certain cancers (Nadeem, 2010). Most cereals and legumes are rich in protein and fat but they have antinutritional factors which discourage their use in food. One such effect is phytic acid (*myo*-inositolhexakisphosphate) in these produces. The phytic acid acts as an antinutrient due to its chelation of various metals and binding of protein therefore diminish the bioavailability of proteins and nutritionally important minerals (Bing, 1998) ; (Hurrell, 2003). The other component of cereals as antinutritional factor is tannins which bind to proteins, carbohydrates and minerals and thus reduce digestibility of these nutrients. To reduce these negative effects, decortication, fermentation, germination and chemical treatments are used (Amir, 2009).

Some practical and relatively inexpensive procedures such as soaking, germination, and fermentation, hydrothermal cooking are reported to improve phytate degradation (Frontela, 2011). In general, lower pH, a longer fermentation time, and a higher yeast addition result in a more intensive degradation of phytic acid (Lee, 2003; Tomaz, 2009). Cooking improves the protein quality by destruction or inactivation of the heat-labile antinutritional factors (Gregory, 2010).

2.3.3.1 Phytic acid (Phytate)

Phytate, (*myo*-inositol hexaphosphate), is a naturally-occurring compound found in all seeds and possibly all cells of plants (Barabara, 1995). It accounts for 60 - 90% of the total phosphorus content of cereals. In cereal and grains such as rice, sorghum and wheat, phytate is deposited together with protein and minerals in the outer layer or, as are the case for maize, in the germ. In

legumes and seeds, phytate is deposited in the cotyledons and embryo (Lee, 2003). Phytate may serve many important physiological functions during dormancy and germination of seeds. These include the storage of phosphorus, high-energy phosphoryl groups and cations. Phytate content in fruits and vegetables is generally lower than in cereals (Lorri, 1997). In its native state it complexes with proteins as well as mono- and divalent cations and is consumed by humans and animals chiefly in cereal grains and legumes or foods derived from them (Barabara, 1995). Phytate are the principal storage form of phosphorus. These chelate divalent cations such as calcium, magnesium, zinc and iron, thereby also reducing their bioavailability (Nadeem, 2010). Phytic acid has often been considered as an antinutrient due to its ability to bind minerals and proteins, either directly or indirectly, and thus change their solubility, functionality, absorption, and digestibility (Tomaz, 2009).

Most phytic acid-mineral complexes are insoluble at physiological pH, which is the main cause of the poor bioavailability of the mineral complexes. The stability of the mineral complexes depends on the number of phosphate groups on the inositol ring. Weaker complexes are formed with lower inositol phosphates. Phytases are enzymes that catalyse the degradation of phytate to lower inositol phosphates and free inorganic phosphorus, depending on the extent of the enzyme activity (Tomaz, 2009).

2.3.3.1 Tannin

Tannin is astringent polyphenolic compounds widespread in plants. Tannin will cause decreased feed consumption in animals, bind dietary protein and digestive enzymes to form complexes that are not readily digestible (Oyewole, 2007), and there is substantial evidence that plants are protected from herbivory using condensed tannins as prevention. However, tannins may also have positive benefits including anti-oxidant properties and potential protection from some types of intestinal pathogens (Rothman, 2009). These naturally occurring plant polyphenols combine with proteins and other polymers such as cellulose, hemicellulose and pectin, to form stable complexes. The number of functional groups in one molecule of tannin dictates its essential properties: formation of complexes with proteins, formation of chelates with metal ions, and other reducing capacities (Riestraa, 2010).

There are certain methods to be followed in order to remove condensed tannins include dehulling the seeds to remove the tannin rich outer layer, autoclaving or treatment with alkali observed a reduction in the tannin content of after fermentation with lactic acid bacteria. The

treatment of tannin containing feeds with oxidizing agents and supplementation with a tannin-complexing agent, polyethylene glycol, could mitigate their negative effects on animals (Naiyat, 2009). Cooking reduce the tannin content of foods (Awadelkareem, 2009).

2.4 Injera

Injera is thin, fermented Ethiopian traditional bread made from flour, water and starter (ersho) which is a fluid saved from previously fermented dough. *Teff* is ground into flour, fermented for three days then made into *injera*. *Teff* is free in gluten and therefore, the bread remains quite flat (Zegeye, 1997). When eaten in Ethiopia, *teff* flour is often mixed with other cereal flours, but the flavor and quality of *injera* made from mixtures is considered less quality interms of its sensory attribute and taste. *Injera* made entirely from barley, wheat, maize or millet flours is said to have a bitter taste. The degree of sour taste is imparted by the length of the fermentation process. If the dough is fermented for only a short period of time (no more than ten days), *injera* has a tasty sweet flavor. The micro-organism primarily responsible for the fermentation process used to make *injera* have indicated that the yeast, *Candida guilliermondii* (Gamboa, 2008). The major quality attribute of a good *injera* is its slightly sour taste. It has been reported that normal and typical *injera* is round, soft, spongy and resilient, about 6 mm thick, 60 cm in diameter with uniformly spaced honeycomb-like 'eyes' on the top (Zegeye, 1997). *Injera* from *teff* flour had white top and white bottom surfaces; many small evenly spread eyes, very soft texture and a bland aftertaste (Yetneberk, 2005) .A higher number of larger eyes is a very desirable attribute of an attractive *injera* (Ashenafi, 2006).Its surface has essentially evenly spaced gas holes, that make up a honeycomb-like structure formed due to the production of gas during fermentation and baking. The bottom surface of *injera* is smooth and shiny. A good *injera* is soft, fluffy and able to be rolled without cracking. It should retain these textural properties after two to three days of storage, which is traditionally done in a straw basket. A slight sourness is a characteristic taste of *injera*. Because *injera* is leavened bread made from nongluten containing flour, it has great potential for commercial production internationally (Yetneberk , 2004).

2.5 Traditional injera processing

Injera can be produced from any of the various cereals depending on availability and abundance of the cereals, which are cultivated in the agro-ecological zones suitable for their growth. Generally speaking, people on the highlands prepare *injera* from barley and wheat whereas those

on the lowlands prepare it from maize, sorghum or millet. Wherever the soil type and rainfall patterns are suitable for cultivation of *teff*, *Injera* from *teff* is more favored than that from the other cereals (Taylor, 2004)

The first 18 hours after mixing flour, water and starter are characterized by vigorous evolution of gas and maximum dough-rising (Fig.1). This is followed by the appearance of an acidic yellowish liquid on the surface of the dough at about 30-33 hours of fermentation. Gas evolution decreases after the pH has fallen below 5.8 (31 hours). The liquid layer is discarded at the end of the first stage of fermentation. As soon as the liquid layer is poured off, about 10 % of the fermenting dough is mixed with three parts of water and boiled for 2 to 5 minutes. This is called '*absit*', a dough enhancer, and it is mixed with the rest in the fermentation vat. This process signals the initiation of the second stage of fermentation. By mixing the boiled dough with the rest in the vat, the dough-rising and gas formation processes are enhanced so they occur in a short time. Maximum dough-rising, which normally takes 30 minutes to 2 hours, signals the termination of fermentation. At this stage the fermenting dough is thin enough to pour onto the hot flat pan, locally known as '*mitad*' for steam-baking into *injera* (Ashenafi, 2006).

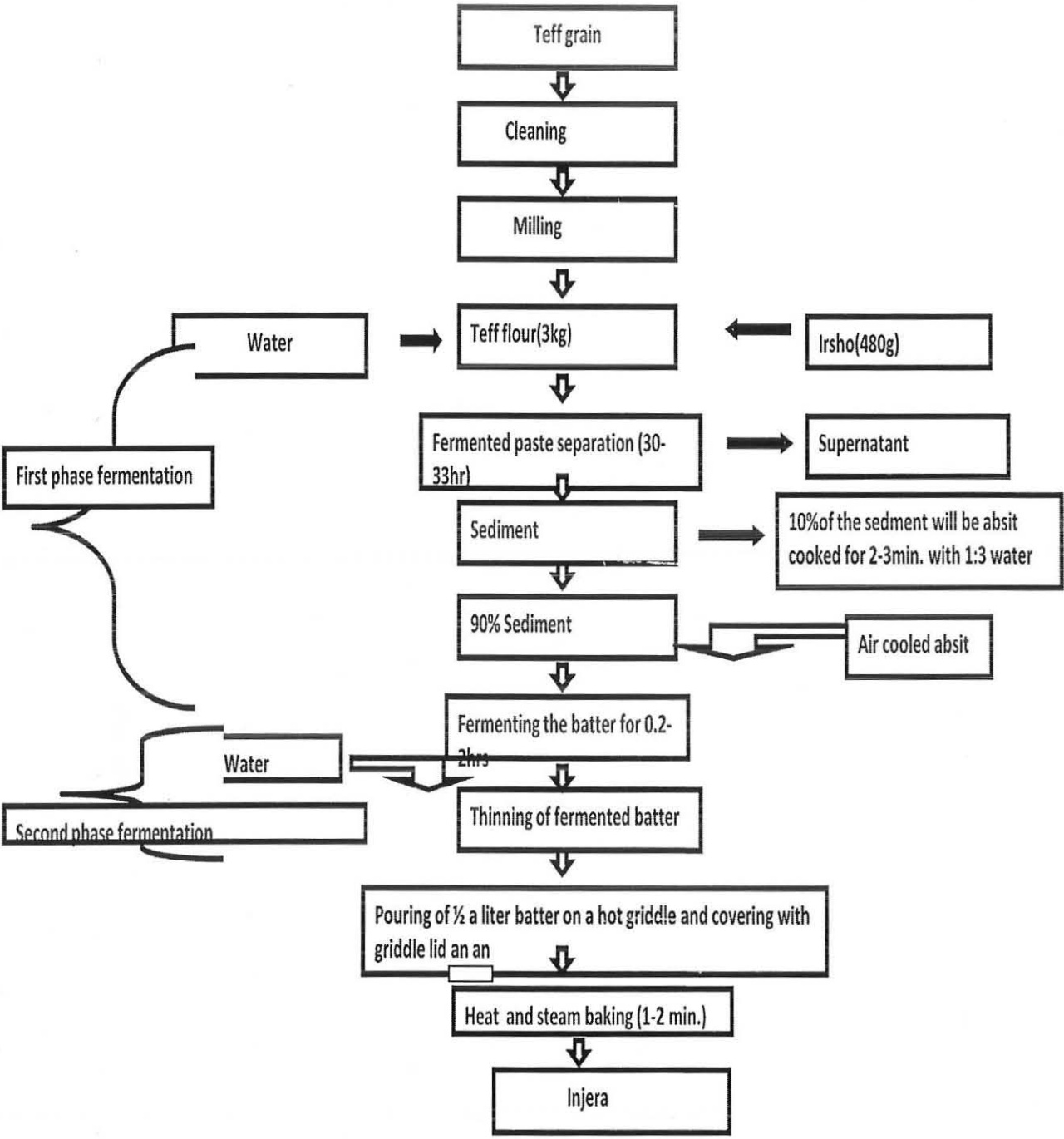


Figure 1 Flow diagram for the preparation of injera (adapted from Ashenafi,2006 ;Beuchat, 1983)

On the basis of production procedures three types of *injera* are distinguishable: thin *injera* which results from mixing a portion of fermented sorghum paste with three parts of water and boiling to yield a product known as '*absit*' which is, in turn, mixed with a portion of the original fermented flour ; thick *injera*, which is reddish in color with a sweet taste, is a '*teff*' paste that has undergone only minimal fermentation for 12-24 hours; *komtata*-type *injera*, which is produced from over-fermented paste, and has a sour taste. The paste is baked or grilled to give a bread-like product. Yeasts are the major microorganisms involved in the fermentation of the sweet type of *injera* (Beuchat, 1983).

The various *injera* types produced from the different varieties of cereals do not have significant variation in their calorie, moisture, protein, carbohydrate or phosphorus contents. Significant variations are, however, observed in the other nutrients. The fermentation process results in significant reduction of most of the nutrients found in the cereal flour. However, in general, *injera* can be considered as good sources of energy, fiber, iron and vitamins but it needs longer fermentation process and favorable condition for it's the preparation procedure (Taylor, 2004)

2.6 Prefermented flour

Prefermented flour is flour having distinctive flavor and acidic properties which is incorporated into baked products (Hoffman, 1976). The acidic nature of the flour is due to the formation of lactic acid during fermentation. During drying the fermented dough, the acid will be more concentrated. According to Inyang, (2006), the acidic nature of the prefermented flour gives it more shelf stable property than unfermented flour.

2.6.1 Prefermented flour preparation steps

In Preparing prefermented flour and substantial amounts of water are mixed together and fermented with microorganisms' responsible for fermentation of the product and then dried so as to provide finely divided flour. The ingredients in bakery products processing are unfermented flour, salt, and yeast, should be mixed in an appropriate sized mixer. Water should be added and the ingredients should be mixed at low speeds until all the ingredients incorporate together. Starter or yeast should be used to ferment flour by mixing it with flour and water followed by maintaining the mixture for a period of time at a suitable temperature for fermentation. The ratio

of flour to water is at least about 2 to 3 to about 4 to 1. After the fermentation is completed, flour is then dried so as to produce finely divided flour (Hoffman, 1976).

2.6.2 Use of the prefermented flour

The fermented flour can then be used to make bakery products having distinctive flavor and acidic properties, by means of the "no time dough" process. This is an improvement over the sponge method in that it eliminates the necessity of user having to repeatedly ferment a sponge, thus saving the baker much time and labor. Sour flour, unfermented flour, salt, yeast and shortening, if used, should be kept in an appropriate sized mixer. Water should be added and the ingredients should be mixed at low speeds until all the ingredients incorporate together. Slight variations in the amount of high gluten flour may be required to obtain the proper consistency of the dough. The ingredients should be mixed well. The dough should be rested for 30 minutes at room temperature and scaled off to the desired weight and baked (Hoffman, 1976).

2.6.3 Advantages of prefermented frozen dough and prefermented flour

Baking has traditionally been a highly labor intensive business. This together with low product prices, and substantial equipment investments, especially in bread making, keep profit margins low. The low profitability of baking is further exacerbated by the necessity to operate during night hours so that fresh products will be available when supermarkets open in the morning. Most customers nevertheless do their shopping after working hours, when bread has already staled for at least 12 hours. This kind of production also places considerable demands on transportation, as products should be moved to the retailer as soon as possible after production. If customers are to obtain fresh products and producers higher profits, baking times must be extended. A most interesting solution for this is frozen doughs, (Rasanen, 1998) and Prefermented flour (Hoffman, 1976) the use of which has increased.

The use of frozen doughs has been of great interest since the 1960's and they are now widely used in industrial bakeries. However, loaf volumes are usually smaller and quality is poorer for breads baked from frozen doughs than in fresh baking. This is especially seen for doughs with low fat content (Rasanen, 1998).

A common finding is that prolonged frozen storage of dough leads to a reduced volume of bread. The deterioration of the baking performance of frozen dough has been ascribed to the loss of

yeast viability, to changes in the gluten fraction due to the liberation of reducing compounds from yeast cells, and to the formation of ice crystals. In general, freeze damage is more pronounced when dough is frozen after proofing and transferred to the oven in the frozen state. In one case, the porous structure is affected by the solubilization of CO₂ at low temperatures and by mechanical damage of the gas cell membrane caused by ice crystals. In addition, the growth of ice crystals in the gas pores contributes to a redistribution of water in dough. (Baier-Schenk,2005).

Prefermented flour can be used to manufacture uniform sour dough bakery products such as sour dough bread, sour dough biscuits, pancakes, rolls, sour rye bread, sour dough English muffins, soda crackers and variants such as oyster crackers, cracker meal, etc. Thus, when the fermented flour is combined with unfermented flour and is baked, the flavor and acidity of the fermented flour is retained, so that the baked product has the flavor and acidity characteristics of sour dough products prepared by the sponge process. In general, the weight ratio of fermented flour to untreated flour in the dough formulation is in the range of 1:9 to 9:1 depending on the acidity and flavor desired (Hoffman, 1976).

2.7 Different drying methods used for food products

Drying is a very important aspect of food processing. The main function of drying is to lower the water activity (a_w) of the product (Devahastin & Sachin Jangam, 2011). Microorganisms that cause food spoilage and decay cannot grow and multiply in the absence of water. Also, many enzymes that cause chemical changes in food and other biological materials cannot function without water (Dominguez, 2011). Consequently, reducing the water content of a food product not only eliminates the opportunity for microbial deterioration but also reduces the rates of other deteriorative reactions, decrease chemical reactions in order to prolong the shelf life of the product at room temperature. It also results in less space needed for storage and lighter weight for transportation (Sudathip, 2010).

Drying and dewatering impact the mechanical, sensory and nutritional properties of food products, and can be used to create new functionalities. Drying is one of the main techniques for preserving agricultural and food products; it takes place in the processing of many products, as the main operation or as a consequence of other processing steps. Heat and mass transfer

phenomena which are typical of drying also appear during other processes, as in cooking, baking, roasting, smoking, refrigeration, freezing, and storage (Elisabeth, 2011).

In the food industry, drying is applied to many different types of products such as low hydrated agricultural products, as a complementary drying for stabilization, highly hydrated agricultural products, for weight reduction and stabilization, and for absorbing the seasonal character of some productions, intermediate products from industrial processes, for stabilization and conditioning and industrial by-products for feed (sugar beet pulps, brewery spent grains, meat or fish meal, whey) and it has many purposes (Elisabeth, 2011).

Dried foods must be processed with the goal of maintaining their quality, such as flavor, texture, convenience, and functionality, increasing their nutritional content, and reducing anti-nutritional factors or toxins. Frequently, there is an extreme focus of food processing on maintaining the bioactivity and structural functionality of the product (Elisabeth, 2011). Many biochemical reactions can be induced by temperature increase in foods: Millard reactions, vitamin degradation, fat oxidation, denaturation of thermally unstable proteins (resulting in variation of solubility or of the germinating power of grains, for example), enzyme reactions (which can either be promoted or inhibited), and so on. Some of these biochemical reactions generate components suitable, for example, for their sensory properties (flavor development); others may be more or less undesirable for nutritional or potential toxicity reasons (vitamin losses, changes in color, taste or aroma, formation of toxic compounds). All the reactions are linked to the simultaneous evolution of product composition, temperature and water content (or chemical potential, or water activity), these factors varying differently from one point to another, from the center to the surface of the products (Elisabeth, 2011). By choosing suitable drying methods and the appropriate conditions, the final product quality can be controlled.

2.7.1 Sun drying

Sun drying is probably the oldest practice and is still popular in some parts of the world today (Chen, 2008). Sun-drying represents a low cost processing method of preserving agricultural products in the tropics (Jimoh, 2009), (Bankole, 2005). Traditional sun drying takes place by storing the product under direct sunlight. Sun drying is only possible in areas where, in an average year, the weather allows foods to be dried immediately. The main advantages of sun drying are low capital and operating costs and the fact that little expertise is required,

contamination, theft or damage by birds, rats or insects; slow or intermittent drying and no protection from rain or dew that wets the product, encourages mould growth and may result in a relatively high final moisture content; low and variable quality of products due to over - or under-drying; large areas of land needed for the shallow layers of food; laborious since the product must be turned, moved if it rains; direct exposure to sunlight reduces the quality (color and vitamin content) of some fruits and vegetables. Moreover, since sun drying depends on uncontrolled factors, production of uniform and standard products is not expected. The quality of sun dried foods can be improved by reducing the size of pieces to achieve faster drying and by drying on raised platforms, covered with cloth or netting to protect against insects and animals. (Fellows, 2000)

2.7.2. Freeze drying (Lyophilization)

Freeze Drying is a process which extracts the water from foods and other products so that the foods or products remain stable and are easier to store at room temperature (ambient air temperature). Lyophilization is carried out using a simple principle of physics called sublimation. Sublimation is the transition of a substance from the solid to the vapor state, without first passing through an intermediate liquid phase. To extract water from foods, the process of lyophilization consists of: freezing the food so that the water in the food becomes ice; under a vacuum, sublimating the ice directly into water vapor; drawing off the water vapor; once the ice is sublimated, the foods are freeze-dried and can be removed from the machine (Fellows, 2000).

The drying of food products via freeze drying has two main characteristics: the virtual absence of air during processing (the low processing temperature and the absence of air prevent deterioration due to oxidation or chemical modification of the product), and drying at temperatures lower than the ambient temperature (products that decompose or undergo changes in structure, texture, appearance, and/or flavor as a consequence of high temperature can be dried under vacuum with minimum damage (Dominguez, 2011).

Freeze-drying is the drying method that gives final products of the highest quality compared to other drying methods. Due to the absence of liquid water and to the low temperatures required for the process, most deterioration reaction rates are very low, which gives a final product of excellent quality. The solid state of water during freeze-drying, with restricted movement in comparison to liquid water, protects the primary structure, and preserves the original structure

and the shape of the food material with minimal reduction in volume .However, it requires a rigorous control of state transitions of both water and solids during dehydration and of dried solids during storage (Elisabeth, 2011).

2.7.3 Oven drying

Oven drying is one of the most frequently used operations for food dehydration. It is the most widely used method to produce dried foods and agricultural products due to the low investment and operating cost. It is a method in which heated air is blown over food materials with the aid of fan(s) to remove most of the moisture from the food material (Jimoh, 2009) . However, a disadvantage of hot-air drying is that it takes a long time, even at high temperature, which in turn may cause serious damage to the product's quality attributes, such as flavor, color, texture, nutrient status and beneficial substances to health (Sudathip, 2010). The drying of wet materials induces a number of physico-chemical changes in the product, often reflected by color (Jimoh, 2009) .

2.7.4 Spray drying

Spray drying is a method of producing dry powder materials from a liquid or slurry by rapidly drying with a hot gas, generally air, that is, 150–200 °C, by atomizing the liquid or slurry at high velocity and directing the spray of droplets into the flow of hot air. In some cases, other gases, such as nitrogen, can be used if the product is oxygen sensitive or the liquid is flammable. Spray drying allows a quick heat-and-mass transfer and produces a high-quality product that, after reconstitution, closely resembles the original product and is economical to manufacture. The atomized droplets have a very large surface area in the form of millions of micrometer-sized droplets (10–200 μm), which results in a very short drying time when exposed to hot air in a drying chamber (Dominguez, 2011) .

Spray drying process, is a unique process in which particles are formed at the same time as they are dried. It is very suitable for the continuous production of dry solids in powder, granulate or agglomerate formed from liquid feed stocks as solutions, emulsions and pump able suspensions. The end product of spray drying must comply with precise quality standards regarding particle size distribution, residual moisture content, bulk density, and particle shape (Peighambardoust, 2011).

The most important advantages of spray drying are: the drying process is performed at low temperatures; the drying process is very short (less than 30 s); and the product is of excellent quality with no adverse effects (Dominguez, 2011). Depending on the material and application, different spray dryers can be used in processes carried out operating in co-current, countercurrent flow, or a mixture of both process under an angle in the same equipment. The industrial techniques include spray drying with centrifugal or pressure atomization, and two-stage or three-stage spray drying. It is reported that spray drying is effective in drying lactic acid starter cultures where viable microorganisms are expected in higher amount from the dried product. It is important to maintain the metabolic activity of microorganisms responsible for fermentation following the spray drying process. There are evidences that show some strains of lactic acid bacteria could be spray dried without a considerable loss of viability and activity (Peighambardoust, 2011).

2.8 Leavening agents

2.8.1 Microbial leavening

Saccharomyces cerevisiae is the most common yeast used in bread making. Yeast cells metabolize fermentable sugars (glucose, fructose, sucrose and maltose) under anaerobic conditions producing carbon dioxide as a waste product, which acts as a leavening agent and enhances dough volume. Yeast also supports both gluten network and aromatic compounds production (Kessoglou, 2003). Leavening by yeasts is a common practice in bakery processing. The leavening of dough during bread production is as a result of carbon dioxide produced by the fermenting organism which is usually *Saccharomyces* Species. Yeast, specifically *Saccharomyces cerevisiae*, converts the fermentable sugars present in the dough into carbon dioxide. This causes the dough to expand or rise as the carbon dioxide forms pockets or bubbles. When the dough is baked it "sets" and the pockets remain, giving the baked product a soft and spongy texture (Obakpolor, 2010).

2.8.2 Chemical leavening

Bakery foods that depend on chemical leavening systems for their appearance, texture, and taste are relatively recent developments. Whereas yeast leavened products have nourished humans for over 4000 years, chemically leavened products have been available for less than 200 years. There

is wide variety of chemically leavened products. Some are sweets like cakes, doughnuts, and biscuits, whereas others are blander, like, biscuits and many types of crackers (Bellido, 2009).

The word 'leaven' comes from the Latin word 'levare' or 'levo,' which means 'to raise' or 'to make light by aeration.' Leavened or aerated baked foods can be leavened chemically or with microorganisms (Hoseney, 1987). Chemical leavening systems can leaven bread dough faster, more consistently and often times more conveniently than yeast because they do not require lengthy activation periods or stringent environmental conditions for evolving gas. The chemical nature of chemical leavening systems makes them capable, once incorporated into the dough, of producing carbon dioxide in a typical processing or storage conditions such as at high extrusion temperatures, in microwave fields, and following prolonged refrigeration or frozen storage (Bellido, 2009). One major disadvantage of chemical leaveners, though, is that they are unable to duplicate the distinctive flavor imparted to bread by yeast. This undesirable effect can be mitigated by reformulation with other flavored bakery ingredients, or chemical leavening systems may be used in conjunction with yeast. Chemical leavening systems and fermentation temperature affords good control over the production of carbon dioxide during dough leavening. Better control over gas production should then translate into better control over the quality of the bread. In chemical leavening, the leavening acid neutralizes a bicarbonate salt in the presence of water, to produce carbonic acid that quickly decomposes into carbon dioxide and water (Bellido, 2009).

The leavening acids are added in their powdered form as salts which do not react until they dissolve in water. The filler stabilizes the product by keeping the baking soda and leavening acid separate and standardizes it to the desired strength. Leavening acids are selected primarily on the basis of reactivity how fast they react and at what temperatures. Reactivity depends mostly on solubility, which in turn depends on chemical composition, particle size, and special treatments such as coating. Single-acting baking powders contain a single leavening acid and can be slow acting or fast acting. Slow-acting types are the most common and use a slow-acting acid like sodium aluminum phosphate that reacts very little until heated in the oven. Fast-acting types are less common but use a fast-acting acid such as monopotassium tartrate (cream of tartar) to provide gas production at low temperatures immediately after addition. Double-acting baking powders contain a mixture of a fast-acting leavening acid like monocalcium phosphate monohydrate and a slow-acting leavening acid like sodium acid pyrophosphate. They react

partially at low temperatures and partially at high temperatures to provide uniform leavening throughout processing. Taking all into consideration we could say that the key to superior chemical leavening is the selection of the correct type and grade of bicarbonate and acid for the baked goods and the baking process being used (Gabriela, 2007).

Sodium bicarbonate (Baking Soda)

The heat of baking can cause baking soda to decompose, giving off carbon dioxide leavening gas without reacting with a leavening acid. The sodium carbonate formed by the decomposition of baking soda is very alkaline and will tend to give the baked product a high pH. This is desirable in some applications, because the high pH enhances certain flavors, such as chocolate. Many recipes for chocolate chip cookies call for leavening only with baking soda (Vetter, 2003).

Cream of tartar

Cream of tartar (potassium acid tartrate) reacts very rapidly with soda in a dough or batter, releasing 70–80% of the leavening gas within 2 min of completion of mixing. For this reason, it has little application in commercial production of bakery foods, since there is insufficient time to process the dough prior to baking (Vetter, 2003).

Sodium acid pyrophosphates

Sodium acid Pyrophosphates are available with a range of reaction rates from slow to very slow. Sodium acid pyrophosphates are used Sodium aluminum phosphates almost exclusively in some applications such as cake doughnuts and canned, refrigerated biscuit dough. They are one of several options available to the formulator of cakes, biscuits, pancakes, etc. after taste limit the use of Sodium acid pyrophosphates (Vetter, 2003).

Baking powder

Baking powder contains baking soda, and one or more leavening acids, and a filler. The leavening acids are added in their powdered form as salts which do not react until they dissolve in water. The filler stabilizes the product by keeping the baking soda and leavening acid separate and standardizes it to the desired strength. Leavening acids are selected primarily on the basis of

reactivity, how fast they react and at what temperatures. Reactivity depends mostly on solubility, which in turn depends on chemical composition, and particle size (Gabriela, 2007).

3. Materials and method

3.1 Study site

Experiments were carried out in the Laboratory of the Food Science and Nutrition Program, at the college of Natural Sciences, Addis Ababa University and Ethiopian Health and Nutrition Research Institute (EHNRI). Addis Ababa is the capital city (AAU) of Ethiopia with the altitude of 2444 m above sea level with a mean annual rainfall of 1196mm and located 9°01 N and 038°45 E. The minimum and maximum temperatures were 9.9°C and 24.6°C, respectively (Fissha Itana & Olsson, 2004).

3.2. Description of the Variety of Teff Used for Analysis

According to Kebebew (2011) Quncho (DZ.Cr-387) was predicted to be in a few years the dominant tef variety grown in the high potential major tef-growing areas of Ethiopia. It is a very white variety. The tef variety Quncho was developed by Debre Zeit Agricultural Research Center (DZARC). The ovule parent DZ-01-974 (Dukem) is a high-yielding variety developed in 1995; however, because of the seed color (pale white), its preference by farmers was very limited. On the other hand, the pollen parent DZ-01-196 (Magna) is an old improved variety developed by pure line selection and released in 1970. DZ-01-196 has been popular for its very white seed color, but its productivity has been relatively low. Hence, a targeted cross was made between the two varieties, with the objective of selecting recombinants combining the high yield of DZ-01-974 and the seed quality trait of DZ-01-196. Quncho was then developed after series of multi-environment yield tests in various major tef-growing regions of the country, was officially released in 2006.

3.3 Procurement of materials

The *teff* variety used for analysis was *Quncho (DZ.Cr-387)* brought from Debrezeit Agricultural Research Center (DZARC). Chemicals used for analysis were obtained from Addis Ababa University food science and Nutrition Laboratory. All the equipments were cleaned and free from any possible contaminants prior to every analysis.

3.4 Sample preparation procedures

The *teff* sample brought from DZARC was cleaned with traditional methods. The grain was milled in a commercial miller house with a great care of contamination and sieved in a 0.425mm sieve and stored in polyethylene bag, for further analysis.

3.3.1 Preparation of prefermented flour

3.4.1.1 Preparation of dough

Twelve plastic containers (three for the drying methods and the other three for the control sample) were washed and dried. 300gm flour and 600ml of water were mixed with 100ml of back sloped liquid starter culture Fig.2. The back sloped starter culture used was prepared from the flour which was used for analysis to avoid contamination. The mixture was kneaded for five minutes equally in all of the 12 containers. The dough was left to ferment for 48 hours. The amount of water used for the dough preparation was done from the experience of my aunt (W/ro Mistere Alemayehu) because the amount of water needed depends on the variety of teff used. The yellowish liquid at the top of the dough was discarded after 48hrs fermentation (Ashenafi, 2006). After the liquid is discarded the dough was measured in a graduated cylinder and 1/10 of it was added in a boiling water and cooked for 2-5min. We call it "*absit*". The water to dough proportion of *absit* was 3:1 (Ashenafi, 2006). After the *absit* was cooled it was mixed with the rest of the dough. Then it was left to ferment once again and it took 35min to rise. The dough which is ready to be baked in the regular process was taken into different dryers. And part of it was baked for control.

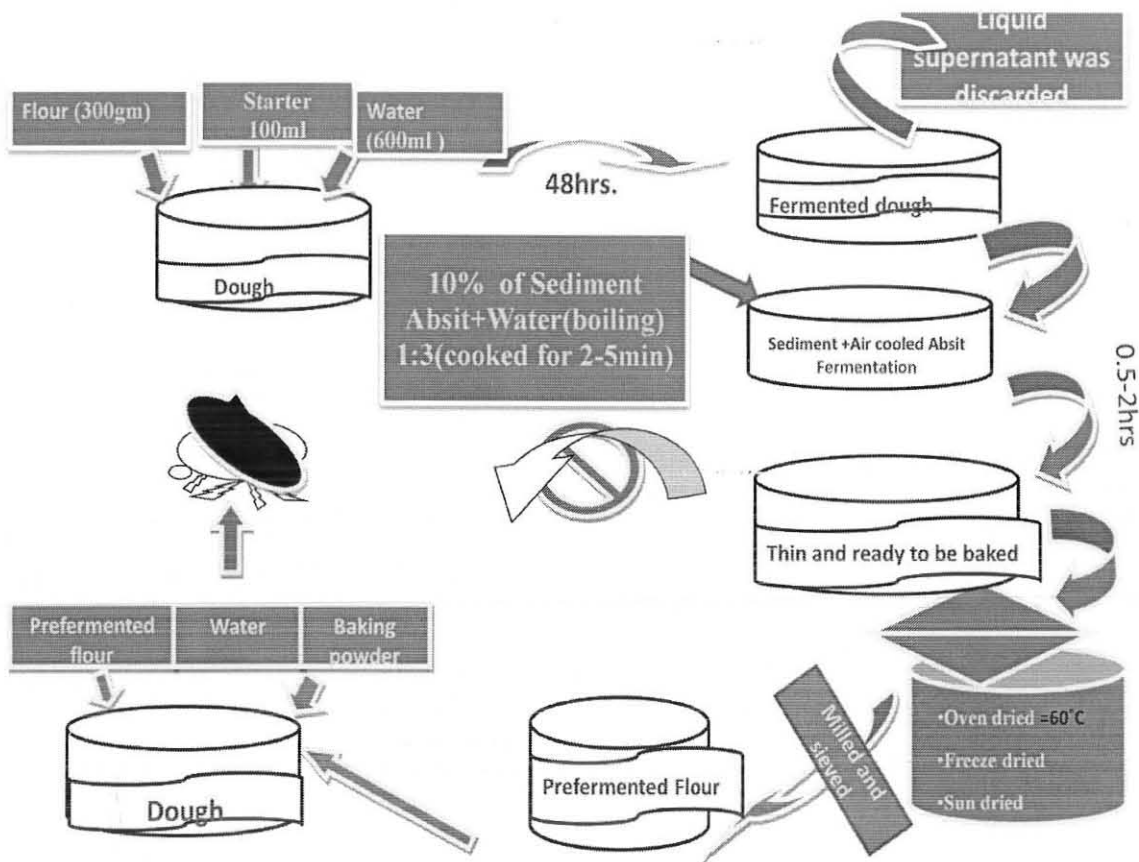


Figure 2 Flow diagram of process followed in injera preparation from prefermented instant flour

3.3.1.2 Preparation of prefermented flour

3.3.1.2 .1 Preparation of freeze dried prefermented flour

The fermented dough prepared in the traditional process was dried with freeze drying equipment. The temperature used in the freeze drier was (-43-47°C). The dried dough was milled again and sieved with a 0.425 mm sieve. The dough was dried within 5 days.

3.3.1.2.2 Preparation of oven dried prefermented flour

The prefermented dough was poured in to metal plates and it was kept in a hot air drying oven with a temperature of 60 °C to avoid further fermentation (Zahouli, 2010). The dried dough was milled again and sieved with a 0.425 mm sieve. The dough was dried within 7 hrs.

3.3.1.2.3 Preparation of sun dried prefermented flour

For sun drying the dough was poured in flat bottomed containers and allowed to dry using sun. In order to prevent the dough from contaminants, it was covered with a thin cloth. The dried dough was milled again and sieved with a 0.425 mm sieve. The dough was dried within 6hrs.

3.3.2 Preparation of *injera* from prefermented flour

First Trial

All the dried products were milled by a laboratory mill and sieved with 0.425mm sieve to get prefermented flour. After having the prefermented flour, it was mixed with cold water and the mixture was left to undergo fermentation and baked after 30 min proofing time (Fig.3).

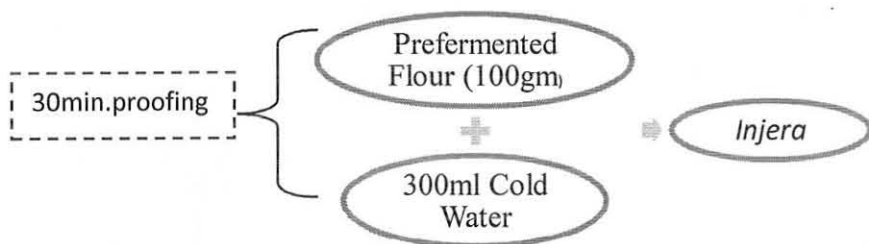


Figure 3 *Injera* preparation from prefermented flour with addition of cold water

Second Trial

The second procedure was mixing hot water to the flour used in the first procedure and left to ferment for 30min. and baked (Fig.4).

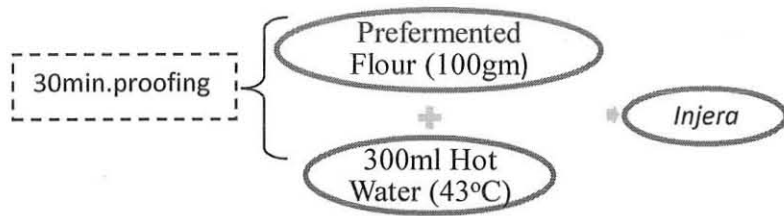


Figure 4 *Injera* preparation from prefermented flour with addition of hot (43 °C) water

Third Trial

The third procedure was adding back sloped starter culture to the flour used in the first procedure and left to ferment for 30min. and baked (Fig.5).

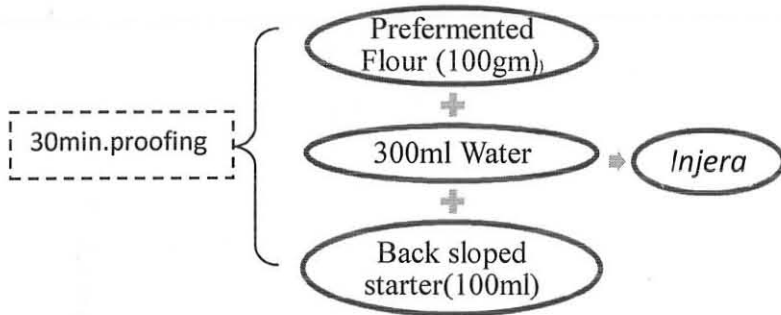


Figure 5 *Injera* preparation from prefermented flour with addition of back sloped starter

Fourth Trial

The fourth procedure was adding "*Absit*" to the prefermented flour and left to ferment for 30min. and baked (Fig.6).

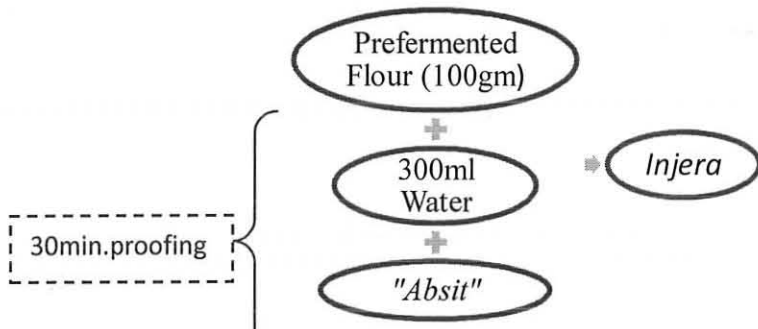


Figure 6 *Injera* preparation from prefermented flour with addition of "*Absit*"

Fifth Trial

The last procedure was mixing prefermented flour with water and left for 20min to rehydrate and baking powder (baking soda and phosphates of sodium) was added and stayed for 10min and baked (total of 30min.) (Fig.7). In all cases dough mixtures were agitated by mixing to ensure that proper eyes are created (Obakpolor, 2010). Prefermented flour prepared in all the three drying methods was used in the four optimization trials.

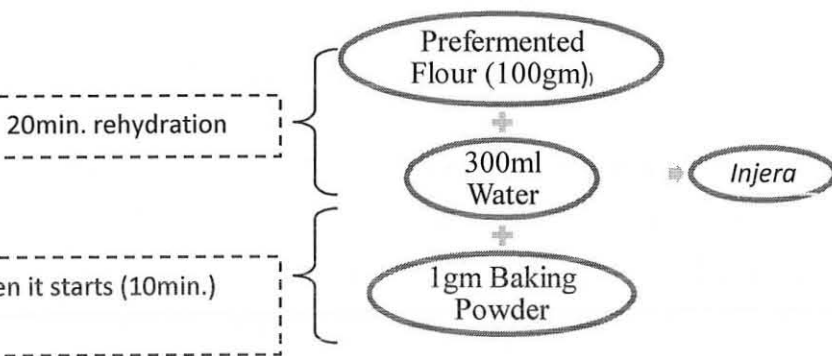


Figure 7 Injera preparation from prefermented flour with addition of baking powder

The samples used for sensory quality and chemical composition analysis were injera prepared from prefermented flour using baking powder.

3.4 Sensory evaluation

Injera prepared from the differently dried prefermented flour with baking powder was evaluated for its sensory acceptability by using 50 consumer panels. All the panelists were frequent consumers of *injera*. The age range of the panelists was 22-45 so that they could fill the score card properly. At the beginning of the taste panelists were instructed about the objective of the study and how they could fill the score card based on their evaluation. The panelists were asked to evaluate the different types of *injera* on the bases of appearance, color, taste, fluffiness, eye size and distribution and overall acceptability based on a nine point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9= like extremely) (Idoko, 2006).

3.5 Proximate composition determination

All the analyses were conducted on a dry weight basis except moisture content which is evaluated in wet bases.

3.5.1 Determination of moisture content

Moisture of the prefermented flours and *injera* prepared from prefermented flour were determined according to AOAC (2000). A crucible was dried in an oven at 105°C for 1h and placed in desiccators to cool. The weight of the crucible was determined. 5.0000g Sample was weighed in the dry crucible dried at 105°C for 3h and after cooling in desiccators to room temperature it was again weighed. The moisture content was determined using the following formula.

$$\text{Moisture content} = \frac{\text{wt.of wet sample} - \text{weight of dried sample}}{\text{wt.of wet sample}} \times 100$$

3.5.2 Determination of crude protein

The crude protein content of the samples was estimated by the Kjeldhal method (AOAC, 1995), in which the samples were digested with a known quantity of concentrated sulphuric acid in the Kjeltex digestion apparatus. The digested material was distilled after the addition of alkali. The released ammonia was collected in 4% boric acid Kjeltex Automatic Distilling Unit. The resultant boric acid containing the ammonia released from the digested material was titrated against 0.1 N HCl. The nitrogen content was multiplied by a factor of 6.25 correlate with the amount of crude protein.

$$\text{Nitrogen (\% w/w)} = \text{NHCl} \frac{(V_s - V_b) \times 14}{W} \times 100$$

Where

V_s = Volume (mL) standard HCl solution used in the titration of the sample

V_b = Volume (mL) standard HCl solution used in the titration of the blank

W = Sample weight

N = Normality hydrochloric acid

14 = The molecular weight of nitrogen.

The protein content was calculated from the equation:

$$\text{Protein content (\% w/w)} = 6.25 \times \%N$$

3.5.3 Determination of ash

Total ash content of injera samples was determined according to AOAC (1995). For the purpose, 2gm of each powdered sample injera were weighed into the ashing crucible, placed on a hot plate under a fume hood and the temperature was slowly increased awaited until smoking ceases and samples become thoroughly charred. The dishes were placed inside the muffle furnace set at 550°C for 4hrs, and was removed from the muffle and then placed in a dissector for 1hr to cool. The amount of ash in the sample was measured from difference in weights and expressed as:

$$\text{Ash content} = \frac{W_2 - W_1}{W_3} \times 100$$

Where, W_1 = weight of fresh sample with crucible

W_2 = weight of dry sample with crucible

W_3 = weight of fresh sample

3.5.4 Determination of crude fat

The crude fat was extracted according to AOAC (2000). The cleaned flask (Cylinder) and boiling chips were dried in the drying oven at 100°C for 1hr, cooled in the desiccators for 30min and weighed. Two grams of sample was weighed in thimble containing fat free cotton. The thimbles were placed in the thimble holders, 50ml of Diethyl ether was poured in the flask, the thimble was immersed in the solvent (in the flask) and heated at 55°C in the fat determinator (SZC-C fat determinator) for 1h, hanged the thimble and heated at the same temperature for 2hrs and then the solvent was recovered for 15 min. The heater was switched off, the flask was dried in the drying oven at 70°C for 30 min, cooled in the desiccators for 15 min and then weighed the flask with the extract. The crude fat was determined using the following formula.

$$\text{Crude fat, percent by weight} = \frac{W_2 - W_1}{W} \times 100$$

Where, W_1 = weight of fresh sample with crucible

W_2 = weight of dry sample with crucible

W_3 = weight of fresh sample

3.5.5 Determination of crude fiber

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

3.5.4.1 Digestion: 1.7000g of fresh sample (w_3) was placed into a 600ml beaker; 200ml of 1.25% H_2SO_4 was added, and boiled gently for 30 minutes while watch glass was placed over the mouth of the beaker. During boiling the level of the sample solution was kept constant with hot distilled water. After exactly 30min. heating; 20ml of 28% KOH was added and boiled gently for further 30min. with occasional stirring.

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

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

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

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

Where W_1 = Weight of crucible with sample after drying

W_2 = Weight of crucible with sample after ashing

W_3 = fresh sample weight

3.5.6 Determination of crude carbohydrate

Total carbohydrates were calculated by difference with the following formula:

$$\text{Total carbohydrates (g/100g dry weight)} = 100 - [\text{g protein} + \text{g crude fat} + \text{g ash} + \text{crude fiber}]$$

3.5.7 Determination of gross energy

Total energy was calculated according to the following equations:

$$\text{Energy (kcal/100g)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g lipid})$$

3.6 Determination of antinutritional factors

3.6.1 Determination of phytic acid

The phytate content in the sample was determined according to the method described by Latta and Eskin (1980), and later modified by Vaintraub and Lapteva (1988). About 0.03 g of fresh samples was extracted with 10 ml 0.2N HCl in a mechanical shaker for 1 hour at a room temperature. The extract was centrifuged at 3000 rpm for 30 minute. The clear supernatant was used for phytate estimation. One ml of wade reagent (containing 0.03% solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.3% of sulfosalcilic acid in water) was added to 3 ml of the sample solution (supernatant) and the mixture was mixed on a vortex for 5 seconds. Absorption readings at 500 nm were taken against a blank sample consisting of 3 ml extracts solution with 2 ml of 0.2 N HCl without wade reagent. Sodium salt of phytic acid (4.5-30 mg/ml) was used as standard for construction of calibration curve (Absorbance = -0.004 phytic acid mg + 0.154, $R^2 = 0.988$).

$$\text{Phytic acid (mg/100g)} = \frac{\text{Absorbance} - \text{intercept}}{\text{Slope} \times \text{density} \times \text{wt} \times 3} \times 10$$

3.6.2 Determination of tannins

Tannin was determined by (Burns, 1971) as modified by (Maxson and Rooney, 1972). 0.5 gram of sample was weighed and mixed with 10 ml 1% HCl in methanol in a screw cap test tube. Then the tube was shaken for 24 hr at room temperature on a mechanical shaker. The solution was centrifuged at 1000 rpm for 5 minute. One ml of supernatant was transferred to another test tube 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). After 20 minutes, the absorbance

of the solutions and the standard solution were measured at 500 nm. Blank sample consisted 1 ml of extract solution with 5 ml of 1 % HCl without vanillin-HCl reagent. (+) catechin (0-60 mg /100 ml) was used as standard for construction of calibration curve (Absorbance = 0.004 (+) - catechin mg, $R^2=0.993$).

$$Tannin \left(\frac{mg}{100g} \right) = \frac{Absorbance - Intercept}{slope \times density \times wt. sample} (dilution factor)$$

3.7 Determination of minerals

All the analysis was conducted on a dry weight basis.

3.7.1 Prevention of contamination

To minimize the risk of contamination, glass wares were washed with 10 % HNO_3 acid and crucibles were soaked with 6N HCl for 24 hours after being washed with detergent and water. All materials were then rinsed with distilled-deionized water and dried in an oven before use.

3.7.1 Digestion of injera samples

Digestion of *injera* samples was carried out by following the dry ashing method (Chen, Zhou, & Qiu, 2009) and (Isildak, Turkekul, Elmastas, & Tuzen, 2004) with some modifications. 2.5 grams of sample was placed in a porcelain crucible and ashed in an oven at $450^{\circ}C$ for 24 hr. Ashed material was dissolved in 2 ml of concentrated HNO_3 , evaporated to dryness, heated again to $450^{\circ}C$ for 4 hr. Samples then was dissolved in 2 ml of concentrated H_2SO_4 , 2 ml concentrated HNO_3 and 2 ml of H_2O_2 , and diluted with distilled-deionized water up to 100 ml after adding 1 % $LaCl_3$ (2 ml). A blank digest was carried out in the same way.

For the element analyses atomic absorption spectrometer was used by setting the appropriate wavelength for each metal. All metal ions (Fe, Zn, and Ca) determination was carried out in an air/acetylene flame mode of the spectrophotometer).

3.7.2 Phosphorous determination

Total phosphorus was determined colorimetrically by the phosphovanadomolybdate method (AOAC, 1990). One milliliter of aliquot was taken from sample digest of the dry ashing into a 100ml volumetric flask and 10ml of molybdate and vanadate solution was added to the samples and the standard (KH_2PO_4) and brought up to 100ml volume with distilled water. The mix was

waited to 10-30min.for color development and read on the absorbance of the blank, sample and standards by spectrophotometer at a wavelength of 460nm. Blank was used to set zero absorbance or 100% transmittance. Calibration curve was prepared for the standards, plotting against the respective phosphorous concentration. The concentration of phosphorous in the samples was calculated from the calibration curve. Potassium dihydrogen phosphate was used (2-12 mg/ml) was used as standard for construction of calibration curve (Absorbance = 0.024 phytic acid mg + 0.04, R²= 0.999).

$$P(\text{ppm}) = \frac{c \times v_1 \times v_2 \times \text{mcf}}{s \times A}$$

Where:

C= P concentration in sample digest read from the curve, ppm.

V₁=volume of the digest

V₂=volume of the dilution

S=weight of the sample

A=aliquot (1ml)

mcf= moisture correction factor

3.8 Phytate and non phytate phosphorous analysis

Phytate phosphorous was calculated by assuming phytate contains 28% phosphorus, i.e. [Phytate P = phytate * 0.28] and accordingly, non phytate phosphorous = total phosphorous - phytate phosphorous.

3.9 Statistical analysis

Complete randomized design was used for statistical analysis. One way ANOVA was used to compare the effect of processing method on proximate composition, antinutritional factor, mineral and sensory quality of injera prepared from prefermented flour. All the experimental results were means \pm standard deviation (SD) of three parallel measurements. Means were separated by LSD, using SPSS Version 15. All analysis was done at significance level of p<0.05.

4. Results and discussion

4.1. Prefermented (instant) flour preparation

In this study prefermented dough was dried by using three different drying methods (sun, oven and freeze drying) in order to prepare prefermented flour. With respect to time, oven drying at 60°C temperature took short time for drying the dough (6hrs) compared to sun drying (7hrs) and freeze drying (5days). Each drying method has resulted in flour with different moisture contents; 10.45g/100g, 11.36 g/100g and 14.01 g/100g for oven, sun and freeze drying respectively. This is in agreement finding of (Hoffman, 1976) < 14 g/100g as moisture content of prefermented wheat flour. Each drying method has also resulted in flour with slight differences in color. The sun and oven dried flour was somewhat darker than flour from freeze drying method. According to Jimoh *et al.* (2009) this might be due to thermal degradation of the originally colorless complex polyphenols (proanthocyanidins and lignins) to colored phenols (anthogaridins) during steaming of the paste. The pH values of the prefermented flour were 3.14, 3.18 and 3.38 for sun, oven and freeze drying respectively. The result is in agreement with the finding of (Hoffman, 1976) reported about 3.2 to 4.1 as range of the pH of prefermented flour prepared from wheat. Prefermented flour with acidic properties was reported to have longer shelf life than unfermented flour by Inyang, (2006) in prefermented casava flour.

4.2. *Injera* preparation from prefermented flour (optimization trials)

Trial one: Addition of cold water

In the present study *injera* was tried to be prepared by mixing the prefermented flour with cold water and allowing the mix to stand for 30min as a proofing time. However *injera* prepared by this process was found to be very poor in its appearance with no eyes which is the major characteristics expected from good quality *injera* (Fig 8). A number of factors might contribute for the failure of *injera* preparation by using this process. One of the factors might be inactivation or non viability of microorganisms responsible for CO₂ production during drying process; Hoffman, *et al.* (1976) also found out that during the preparation of wheat bread from prefermented flour microorganisms were not viable in a required amount for preparation of sour bread. According to Yao (2009) compared to other drying methods, freeze drying has been reported in resulting high viable cells (10⁸ cells/ml immediately after freeze drying). However, in

our study *injera* from freeze dried flour was not different from *injera* from other drying methods that will cause the inactivation or non viability of microorganisms. This might be due to the longer drying time used and poor quality of the drying equipment used in the drying process. Fellows (2000) stated that during slow freezing, ice crystals grow in intercellular spaces and deform and rupture adjacent cell walls. Ice crystals have a lower water vapor pressure than regions within the cells, and water therefore moves from the cells to the growing crystals. Cells become dehydrated and permanently damaged by the increased solute concentration and collapsed and deformed cell structure. On thawing, cells do not regain their original shape and turgidity. The food is softened and cellular material leaks out from ruptured cells.

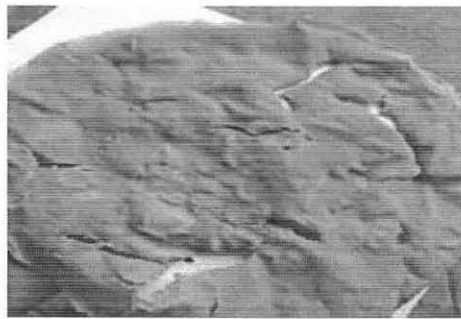


Figure 8 *Injera* prepared from prefermented flour mixed with cold water

A number of optimization trials have been therefore performed in order to prepare good quality *injera* from the (instant) prefermented flour.

Trial two: Mixing the flour with hot water

The first optimization was addition of hot water (43°C) to activate microorganisms found in the prefermented flour. But the quality of *injera* was still poor (Fig.9). The rationale behind adding water heated at 43°C was based on the observation of Poirier (1999) who stated that the optimal rehydration temperature of *S. cerevisiae* is 43 °C. Fellow (2000) was also reported the positive effect of increasing temperature to activate microorganisms. However in our study the use of hot water didn't bring positive result. This might be due to hot water might not be the only factor that promotes the activities of the yeasts. Similarly, Kessoglou (2003) explained that yeast characteristics may also play an important role in determining yeast activation and product quality. Microorganisms might be mesophilic thermophilic or psychrophilic.

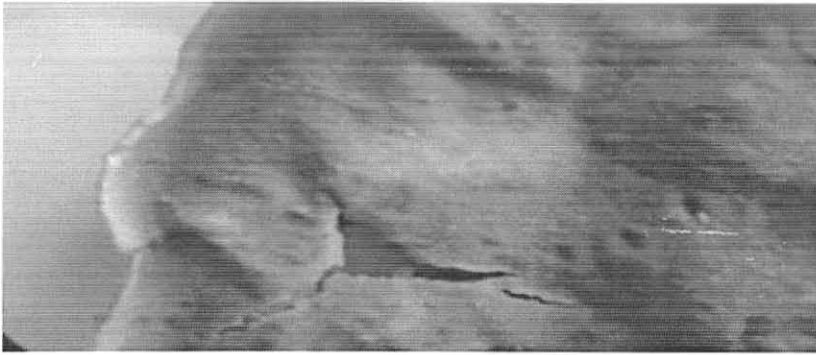


Figure 9 Injera prepared from prefermented flour mixed with hot water (43°C)

Trial three: Addition of “Absit”

The second optimization trial in our study was addition of “Absit” to the prefermented flour to activate yeasts responsible for CO₂ production. After the addition of “Absit” the mix was left 30min proofing time before baking. Ahenafi (2006) stated that the purpose of adding “Absit” during *injera* preparation is to deliver easily digestible starch to the yeasts. This trial was not also successful in our case since the prepared injera was poor (with very few or no eyes) (Fig. 10). This might be due to the microbial cells are highly affected by the drying treatment. As described by Elisabeth (2011) drying limited number of micro-organisms with absence of pathogens. These limited number of microorganisms might resulted in the poor carbon dioxide production for eye formation.



Figure 10 *Injera* prepared from prefermented flour mixed with water and ‘absit’.

Trial four: Addition of back sloped starter culture

The other trial for the preparation of good quality injera from prefermented flour was addition of back sloped starter culture. This trial has resulted in a better injera (with few scattered eyes) compared to the other trials (Fig.11), but the quality of injera was not still satisfactory. The

presence of eyes in this case might be due to the newly added yeasts from the back sloped starter. The proofing time in this trial was 30 min Fellow (2000) described that newly incorporated yeasts might require longer time to produce CO₂. The other reason might be due to the slow growing of microorganisms or digestibility of starch was affected in the drying process.

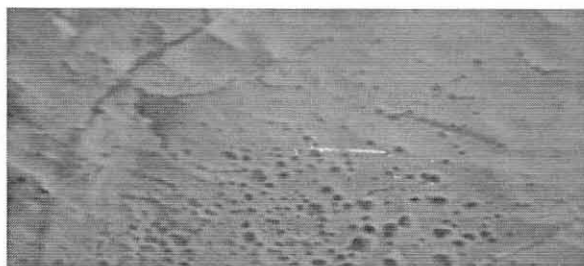


Figure 11 *Injera* prepared from prefermented flour mixed with water and back sloped starter culture

Trial five: Addition of Baking Powder

The last trial in the preparation of good quality injera from prefermented flour was addition of baking powder. This trial was successful since the quality of *injera* prepared was good (with enough number of eyes expected from good quality injera and with proper texture) (Fig.12). Bellido (2009) stated that nature of chemical leavening systems makes them capable once incorporated into the dough, of producing carbon dioxide in a typical processing or storage conditions such as at high temperatures, in microwave fields, and following prolonged refrigeration or frozen storage.

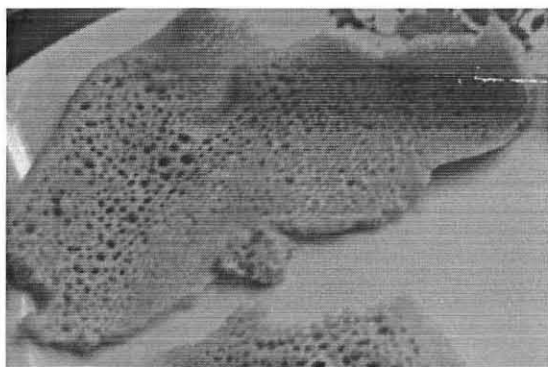


Figure 12 *Injera* prepared from prefermented flour, water and baking powder (10min.).

The samples used for sensory quality and chemical composition analysis were *injera* prepared from prefermented flour using baking powder.

4.3 Effect of processing on sensory acceptability of *injera*

To the consumer, the most important quality attributes of a food are its sensory characteristics (texture, flavor, aroma, shape and color). These determine an individual's preference for specific products, and small differences between products of similar ingredients can have a substantial influence on acceptability (Fellows, 2000). The sensory score for *injera* which is prepared from prefermented instant teff flour is presented in Table 3:

4.3.1 Fluffiness

The mean fluffiness scores of *injera* which were prepared from instant prefermented flour using different drying methods and the traditionally processed ranged from 6.3 - 7.7 (Table 3). Fluffiness score of freeze dried was not significantly different from control sample. The control sample was significantly different from sun dried and oven dried. The sun dried and oven dried has lesser score on fluffiness. The low fluffiness score of *injera* from sun dried and oven dried flour might be due to the breakage of bonding force between starch granules during drying with the application of heat (Jimoh, 2009). When starch based foods are heated in an aqueous environment, they undergo a series of changes like gelatinization and pasting. These are two of the most important properties that influence quality and aesthetic considerations in the food industry, since they affect texture (Dixon, 2005). According to Fellows (2000) the loss of texture, which is a broader term than fluffiness, is caused by gelatinization of starch, crystallization of cellulose and variations in the moisture content during drying creates internal stresses. As a result on rehydrating the product absorbs water more slowly. The freeze dried sample had no significant difference ($p < 0.05$) with the control. This is might be due to the texture of freeze-dried foods which is well maintained. Those foods that are dried under optimum conditions suffer less damage and rehydrate more rapidly and completely than poorly dried foods. There is little shrinkage and no case hardening during drying using freeze drier. In addition the open and porous structure of the dried flour allows rapid and full rehydration (Fellows, 2000).

Table 3 Sensory characteristics of *teff injera* prepared from prefermented flour prepared with different drying methods

Drying tech.	Fluffiness	Taste	Eye	Color	Appearance	Aroma	Acceptability
Control	7.58±1.58 ^a	7.02±1.77 ^a	7.7400±1.86 ^a	7.90±1.45 ^a	7.58±1.80 ^a	7.48±1.69 ^a	7.56±1.49 ^a
Sun dried	6.40±1.72 ^b	5.24±2.03 ^c	6.72±1.45 ^b	6.22±1.52 ^c	6.16±1.77 ^b	5.84±2.15 ^c	5.74±2.06 ^c
Oven dried	6.30±1.67 ^c	6.18±1.53 ^b	5.44±1.95 ^c	6.30±1.87 ^b	6.16±2.11 ^b	5.18±2.05 ^b	6.22±1.72 ^b
Freeze dried	7.74±1.44 ^a	6.94±1.98 ^a	7.56±1.59 ^a	7.76±1.45 ^a	7.96±1.19 ^a	7.18±1.83 ^a	7.68±1.17 ^a

*Data is expressed as mean± SD [range] on a dry -weight basis. *Means in the same column followed by different letters are significantly different (P<0.05).*Control represents the traditionally prepared injera

4.3.2 Taste

Taste attributes consist of saltiness, sweetness, bitterness and acidity and some of these attributes can be detected in very low thresholds in foods. The taste of foods is largely determined by the formulation used for a particular food and is mostly unaffected by processing (Fellows, 2000). Sensory scores for the attribute taste in our case ranged from 5.2 to 6.9 (Table 3).There was no significant difference between injera prepared from freeze dried flour and injera prepared by the traditional method (control) (p<0.05). In contrary the control sample was significantly different from sun dried and oven dried. In our study the sun dried sample has got the least acceptability in terms of taste. This might be due to the off odors produced due to the thermal degradation of chemicals or mould growth during drying the sample (Fellows, 2000). The control and freeze dried didn't show significant (p<0.05) difference. This might be due to the effectiveness of the freeze drier to dry and convert foods in to powder without altering their quality even though it is the most expensive method (Mahanom, 1999; Mahendran, 2010).

4.3.3 Eye size and distribution

Eye size and distribution was one of the sensory attributes evaluated in our study. Sensory scores for this attribute ranged 5.4 – 7.7 (Table 3). There was no significant difference in the taste score between *injera* from freeze dried and control $p < 0.05$. The control sample was significantly different from sun dried and oven dried. On the other hand *injera* from oven and sun dried flour has got less score compared to the others. According to Jimoh *et al.* (2009) the reason for the least value given to the sun dried and oven dried might be due to drying of wet materials which induce a number of physico-chemical changes in the product. This might have an impact on the formation of required texture during baking. The freeze dried didn't show significant difference due to the absence of drying temperature applied in the process that will affect the textural properties (Fellows, 2000).

4.3.4 Color

From the consumer-acceptance point of view, color is an important attribute of food products (Chen, 2008). Sensory score for the attribute color in our study ranged from 6.2- 7.9 (Table 3). There was no significant difference between *injera* prepared from freeze dried flour and *injera* prepared by the traditional method. This might be due to the absence of thermal action during freeze drying (Fellow, 2000). However, the control sample was significantly different from sun and oven dried samples. The reason for the less value given to the sun dried and oven dried might be due to drying of foods cause changes in the product. These changes are often reflected by the alteration of color and concentrated as water is removed (Jimoh, 2009). On the other hand, as in hot-air drying, the surface temperature of the product get very high which promotes heat-sensitive chemical reactions such as mallard reactions (Chen, 2008). The other reason might be thermal degradation of the originally colorless complex polyphenols (proanthocyanidins and lignins) to colored phenols (anthogaridins) during steaming of the paste.

4.3.5 Appearance

The result of sensory evaluation on appearance of *injera* which is prepared from prefermented flour of different drying methods (Table 3). It ranged from 6.2-8.0. Appearance scores of freeze dried and control samples gave significantly ($p < 0.05$) higher mean than that of sun dried and oven dried. In contrary, the control sample has been obtained having significantly ($p < 0.05$) higher mean than the sun and oven dried samples. Sun and oven dried samples didn't show

significant difference in their appearance as evaluated by the panelists. The appearance of sun and oven dried samples might be significantly less due to color, fluffiness, and eye size are affected by the thermal treatment during drying(table 4.1). All the treatments got score of >6 which means liked by the consumers.

4.3.6 Aroma

Fresh foods contain complex mixtures of volatile compounds, which give characteristic aromas, some of which are detectable at extremely low concentrations. These compounds may be lost during processing, which reduces the intensity of flavor or reveals other aroma compounds. (Fellows, 2000). The mean aroma scores of *injera* which are prepared from instant prefermented flour using different drying methods and the traditionally processed *injera* ranged from 5.2 - 7.2 (Table 3). Aroma scores of freeze dried was not significantly different from control sample. However, the control sample was significantly ($p < 0.05$) different from that of sun dried and oven dried samples. The higher retention of aroma on the freeze dried sample might be due to the volatile aroma compounds were not evaporated with water vapor produced by sublimation and is trapped in the food matrix. As a result, aroma retention of food products dried with freeze drying is high (Fellows, 2000). The sun dried and oven dried has lesser score on aroma. The reason might be due to application of heat during drying. According to Fellows(2000), heat not only vaporizes water during drying but also causes loss of volatile components from the food and as a result most dried foods have less aroma and flavor than the fresh foods .The sun dried and oven dried samples differ slightly significantly ($p < 0.05$). The slight variation among the sun dried and oven dried on aroma might be due to the extent of loss of volatile compounds. According to Fellows (2000) loss of aromatic compounds depends on the temperature used for drying the food therefore as the applied temperature increase aroma loss increases. All the treatments got score of >5 which means liked by the consumers.

4.3.7 Over all acceptability

The sensory score on overall acceptability in our study ranged from 5.7 - 7.7 (Table 3). The overall acceptability of freeze dried sample was not significantly different from control sample. The control sample was significantly different from oven dried and sun dried samples. The lesser score of sun and oven dried samples might be due to the lesser values for other attributes (color, fluffiness, eye size, aroma, and appearance) are affected by the thermal treatment during

drying. The higher value given to the acceptability of freeze dried agrees with the report of Elisabeth (2011). According to Elisabeth (2011) freeze-drying gives final products of the highest quality compared to other drying methods. Due to the absence of liquid water and low temperatures required for the process, most deterioration reaction rates are very low, which gives a final product of excellent quality. Fellows (2000) also reported that, the solid state of water during freeze-drying, with restricted movement in comparison to liquid water, protects the primary structure, and preserves the original structure and the shape of the food material with minimal reduction in the overall acceptability of the product.

4.4 Proximate composition results of injera processing with different driers

Many unit operations, especially those that do not involve heat, have little or no effect on the nutritional quality of foods. Examples include mixing, cleaning, sorting, freeze drying and pasteurization. Heat processing is a major cause of changes to nutritional properties of foods. For example gelatinization of starches and coagulation of proteins improve their digestibility (Fellows, 2000). The proximate compositions of processing (using prefermented flour) in *teff* injera baking were analyzed. The results of the samples analyzed in this work are presented in the following Table 4.

4.4.1 Moisture content

The mean moisture content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 53.8 to 56.4(g/100g) (Table 4). The moisture content of control sample was significantly ($p < 0.05$) different from freeze dried sample. However, both control and freeze dried samples were significantly different ($p < 0.05$) from oven and sun dried samples. The higher moisture content of freeze dried sample might be due to the little shrinkage and absence of case hardening during drying using freeze drier. In addition the open and porous structure of the freeze dried flour allows rapid and full rehydration (Fellows, 2000). The low moisture content of the sun and oven dried samples might be due to rehydration and moisture holding capacity of starch granules was affected by the drying temperature (Fellows, 2000). The mean moisture content of the control is 58.05 g/100g which is in agreement with the finding of Umeta (2006) (54.3–62.7 g/100g).

Table 4 Proximate composition tef injera prepared from prefermented flour prepared with different drying methods.

Drying tech.	Moisture content* %	Protein %	Ash %	Fat %	Crude fiber%	CHO%	Energy %
Control	58.05±0.23 ^a	8.73±0.65 ^a	2.07±0.08 ^a	4.30±0.56 ^a	1.84±0.07 ^a	83.42±0.79 ^a	405.88±2.48 ^a
Sun dried	53.83±0.62 ^c	8.65±0.66 ^a	3.41±0.38 ^b	2.75±0.20 ^b	2.23±0.24 ^c	82.96±0.69 ^a	391.19±1.88 ^c
Oven dried	53.57±0.25 ^b	10.03±0.21 ^b	3.53±0.13 ^c	3.52±0.18 ^a	1.91±0.09 ^a	81.76±1.99 ^a	386.86±4.75 ^d
Freeze dried	56.4±1.27 ^a	9.05±0.71 ^a	4.65±1.01 ^d	2.62±0.72 ^c	2.67±0.34 ^b	80.25±0.47 ^b	392.82±2.60 ^b

*Data is expressed as mean± SD [range] on a dry -weight basis. *Means in the same column followed by different letters are significantly different (P<0.05). * is expressed in fresh weight base. Control represents the traditionally prepared injera.

4.4.2 Crude protein

The mean crude protein content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 8.7-10.0g/100g (Table 4).The control sample was not significantly(p<0.05) different from sun dried and freeze dried samples. In contrary the control sample was significantly different from oven dried sample. The oven dried sample gave significantly higher mean value than that of the other drying methods this might be due to higher temperature applied during drying (Fellows, 2000). Similarly Akinneye *et al.* (2010) reported that gelatinization of starch and coagulation of protein improves their digestibility. The low crude protein content of injera from sun dried flour might be due to relatively poor control over drying conditions and lower drying rates than those found in other driers, which results in products that have lower quality and greater variability (Fellows, 2000). Crude protein content

of control (8.7) is similar with the report of Bultosa (2007)(8.7-11.1). But had slight variation with Solomon (2007) (9.4–13.3) and Taylor (2004) (9.38-13.3). The slight variations might be due to variety differences of teff used for analysis and fermentation time.

4.4.3 Total Ash

The mean total ash content of *injera* prepared from prefermented flour dried using sun, oven and freeze drying methods ranged from 3.4 - 4.7 (g/100g) (Tables 4). Total ash content of control was significantly different from the other samples prepared from prefermented flour. The higher total ash content of freeze dried sample might be due to the extended time used to dry the sample which might lead to the higher chance of contamination. Similarly the relatively higher value of the sun dried and oven dried from control might be due to contamination during drying and milling processes after fermentation of dough. The ash content of traditionally harvested teff grain is especially high because of grain contamination with the soil during harvest (Refera, 2001) but the sample we took was not traditionally harvested. The total ash content of control was 2.1 g/100g. The result of the control was not in close agreement with the findings of Solomon (2007) (2.7–3.0 g/100g), Taylor (2004) (2.66-3.00) and Bultosa (2007)(2.7-3.0g/100g). The variation between the different reports might be due to variety difference or the way the teff grain was harvested.

4.4.4 Crude fat content

The mean crude fat content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 2.6 g/100g -3.5 g/100g (Table 4). The fat content of control was significantly different from sun and freeze dried samples. However, fat content of control sample was not significantly ($P>0.05$) different from oven dried samples. The low crude fat content of freeze dried sample might be due to open porous structure of dried flour might allow oxygen to enter and cause oxidative deterioration of lipids (Fellows, 2000). The samples prepared with differently dried flour has significantly lower than control. This might be due to lipid oxidation during drying. The fat content of control was (4.3 g/100g), which is not in close agreement with the finding of Bultosa (2002), (2.7–3.0 g/100g), Solomon (2007) , 2.0–3.1 g/100g, Taylor (2004) (2.0-3.1g/100g) and Bultosa (2007). The variation in fat content might be due to the difference in the variety of *teff* used for analysis.

4.4.5 Crude fiber content

Dietary fiber is a physiological and nutritional concept relating to those carbohydrate components of foods that are not digested in the small intestine (FAO, 2003). The result of treatments effects on the crude fiber of processed injera was ranged from 1.9-2.7g/100g for control and freeze dried samples (Table 4). The crude fiber content of control sample was not significantly ($p < 0.05$) different from oven dried samples. The crude fiber content of control sample was significantly different from freeze and sun dried samples. The freeze dried sample gave higher mean value and this might be due to contamination during long drying process. The result found for the fiber content of control (1.84%) has variation with a report of (Solomon, 2007) ranged from 1.98–3.5 g/100g and Taylor (2004) (1.98-3.5g/100g). The slight variation might be attributed to the variety difference in *teff* grain used for analysis.

4.4.6 Crude Carbohydrate

The mean crude carbohydrate content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 81.7-83.0 g/100g (Table 4). The control sample was not significantly ($p < 0.05$) different from sun dried and oven dried samples. However control sample was significantly different from freeze dried sample. The higher carbohydrate content of the control sample may be attributed to the lesser values found in the other nutrients. The freeze dried sample has the lowest carbohydrate content and this might be due to the higher values found in the other nutrients. In the present study the crude carbohydrate content of the control sample was 83.4% is not in close agreement with (Solomon, 2007; Taylor, 2004) 73 g/100g was also reported as carbohydrate content of white *teff injera*.

4.4.7 Gross energy

The mean crude protein content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 386.9-392.8 (Table 4). The Gross energy content of control sample was significantly ($p < 0.05$) different from freeze which also significantly differed from sun dried and oven dried samples. The proximate composition of *teff* flour is given in Table 4. The protein, fat and ash contents of control were found to be 8.7, 2.1, 1.8, (g/100g) respectively. These values are not in agreement with the finding of Solomon (2007) which is reported as (9.3, 2.4 and 2.4 g/100g) respectively. However, the crude fat content of control sample is higher than the fat content reported by Solomon (2007). The variations in fat content

leads to the variation in gross energy content. The lesser value of the oven dried samples might be due to the higher protein content. The mean gross energy content of control was 405.9Kcal/100g, which shows variation with Solomon (2007), who reported 339.9Kcal/100g as crude energy content of white *teff injera*.

4.5 Effect of processing on antinutritional factors

Antinutritional factors are components of food that discourage the use of important food nutrients by the human body. One such effect is chelation of various metals and binding of protein, therefore diminishing the bioavailability of proteins and nutritionally important minerals (Bing-Lan Liu, 1998). The results obtained in antinutritional factor analysis are presented in Table 5.

4.5.1 Phytic acid (phytate)

The mean phytate content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 232.9-264.97mg/100g (Table 5). The phytate content of the control sample was not significantly ($p < 0.05$) different from freeze dried samples. In contrary the control sample was significantly different from sun and oven dried samples. The phytate content of *injera* show a decrease on the oven dried, sun dried, and freeze dried and control respectively. According to Konietzny (2006) temperature applied might be the cause for the more concentrated lactic acid that will lead to the lowering of pH which will improve the degradation of phytate. But the temperature applied might not be the cause for the decrease in phytate content because phytate is heat-stable, significant heat destruction of phytate during cooking is not expected to occur. Umeta (2006) also agrees on the idea that degradation of phytate is higher in lower pH. The mean phytate content of the control value has variation with the result obtained by (Umeta, 2006) which is 273.9mg/100g for white *teff injera*. The result of phytate content in the present study has shown 290.3mg/100g. The slight variation might be due to difference in variety of *teff* used for analysis or/and fermentation time.

Table 5 Phytate and condensed Tannin composition of *teff injera* prepared from prefermented flour prepared with different drying methods.

Drying tech.	Phytate mg/100g	Tannin mg/100g
Control	290.30±16.68 ⁵³⁷ ^a	36.58±6.60 ^a
Sun dried	248.9033±5.96 ^d	38.38±7.49 ^a
Oven dried	232.5533±10.23 ^c	35.43±7.27 ^a
Freeze dried	264.97±21.79 ^a	38.29±3.66 ^a

*Data is expressed as mean± SD [range] on a dry -weight basis. *Any two means in the same column not followed by the same letters are significantly different (P<0.05).* Control represents the traditionally prepared injera.

4.5.2 Tannin content

The mean tannin content of injera which were prepared from instant prefermented flour using different drying methods ranged from 35.4-38.4 mg/100g (Table 5). There is no significant difference in the tannin content of injera prepared from prefermented flour and control sample. According to Elhadi (2003) the attempts of tannin reduction include removal of seed testum and pericarp, inactivation of the seed tannin, the use of alkaline solution, and the use of fertilizers, fermentation, and sprouting. In our study fermentation was the only treatment applied in the products from the use of alkaline solution, and the use of fertilizers, fermentation, and sprouting. However fermentation is applied to all of the samples, which may be the reason for no significant difference among the drying methods used. The tannin content of the control sample

is in agreement with the tannin content reported by (Ahenafi, 2006) see (Table 2.2). The slight variation might be due to the teff variety used for analysis.

4.6 Effect of processing on mineral content

According to Nwachukwu (2007) mineral content as a result of drying was increased. Since minerals are derived from the ash content left after burning off the fresh part of the plant, it is reasonable that drying could increase the mineral contents. It is also possible that drying may have provided a large surface area for increased reaction rate than in the wet analysis. Anaemia, rickets, osteoporosis, and immune diseases are caused by a deficiency of iron, calcium and/or zinc (Frontela, 2009). The results obtained in mineral analysis are presented in the following table.

Table 6 Mineral content of *teff injera* prepared from prefermented flour prepared with different drying methods

Drying tech.	Fe (mg/100g)	Ca(mg/100g)	Zn(mg/100g)	P (mg/100g)
Control	9.46±0.25 ^c	35.08±2.04 ^a	1.51±0.0 ^a	239.90±11.31 ^a
Sun dried	14.18±1.96 ^a	34.92±0.96 ^a	1.63±0.049 ^a	567.85±22.69 ^c
Oven dried	13.27±0.43 ^a	33.65±.78 ^a	1.53±0.07 ^a	659.80±11.31 ^d
Freeze dried	12.12±1.48 ^b	33.62±2.23 ^a	1.60±0.028 ^a	507.85±11.38 ^b

*Data is expressed as mean± SD [range] on a dry -weight basis. *Any two means in the same column not followed by the same letters are significantly different (P<0.05). *Control represents the traditionally prepared injera.

4.6.1 Iron

Iron is an essential trace element whose biological importance arises from its involvement in vital metabolic functions by being an intrinsic component of hemoglobin, myoglobin and cytochromes (Hemalatha, 2007). The mean iron content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 12.1 -14.2mg/100gm

(Table 6). The iron content of control sample was significantly ($p < 0.05$) different from sun, oven and freeze dried samples. The result obtained in the control sample is in agreement with result obtained by Urga (1997). It was reported as 9.2 mg/100gm for the iron content of white *teff injera* (Urga, 1997). But it is in contrast to the results obtained by Refera (2001)(10.4mg/100g). Refera (2001) suggested that *teff* seed is especially high in iron and calcium content. When it is harvested traditionally (15.7 mg per 100 g), this is in part because of grain contamination with the soil during harvest. Therefore the disagreement might be due to our sample, was not harvested traditionally. It was brought from DARI.

4.6.2 Zinc

The importance of zinc (Zn) for growth and development of human beings has been documented widely. Although Zn (and also Fe) from plant sources could be as high as animal sources, bioavailability could be reduced as a result of antinutritional factors. Zn deficiency is suspected to be widespread in populations heavily dependent on cereal-based diets containing few animal products (Karunaratne, 2008). In the present study the mean zinc content of *injera* prepared from prefermented flour ranged from 1.5-1.6 (mg/100g) as shown in (Table 6). There is no significant difference in zinc content between control and sun, oven and freeze dried samples. Our result for the control sample is less than that of the value reported by Urga (1997); 2.3mg/100g. The variation in the zinc content of the samples might be due to contamination during harvesting and *injera* processing or the difference in the variety of *teff* used for analysis.

4.6.3 Calcium

Calcium is an essential mineral required for the diverse physiological and biochemical functions in the human body (Kamchan, 2004). Its deficiency results in osteoporosis in adult and osteomalacia in growing children (Ukoha, 2007). Calcium in association with phosphorous is essential for growth and maintenance of strong bones especially in infants (Fubara, 2011). In the present study the mean calcium content of *injera* prepared from prefermented flour ranged from 33.7-34.9mg/g (Table 6). The calcium content of control was not significantly different from sun dried; oven dried and freeze dried samples. 202.4mg/100g was reported as Ca content of white *teff injera* (Urga, 1997). The difference may be due to the variety of *teff* used for analysis.

4.6.4 Phosphorous

In the present study, mean phosphorous content of *injera* prepared from prefermented flour of different drying methods ranged from 507.9-659.8 (g/100g) (Table 6). The mean phosphorous content of control gave significantly ($p < 0.05$) lower mean followed by sun dried *injera* which also significantly differed from freeze dried and oven dried samples. Three of the samples prepared from prefermented flour showed a higher mean value. This might be attributed to the use of baking powder which contains phosphates of sodium (Noori, 2010). According to Noori (2010) food additives like leavening agents increase the phosphorous content of foods. The mean value of the control sample has a variation with the report of Uрга (1997) 277.21mg/100g phosphorous content of white *teff injera*. The variation might be due to the variety difference.

4.7 Phytate and non phytate phosphorus content

Phosphorous is readily available in a wide range of foodstuffs and bioavailable from several foods. With dietary intakes of 775–1860 mg/d (Kemi, 2010), Common sources of inorganic phosphorus include certain beverages, enhanced or restructured meats, frozen meals, cereals, snack bars, processed or spreadable cheeses, instant products, and refrigerated bakery products. Currently, there is no accurate or reproducible method to distinguish between protein-based organic and preservative- or additive-based inorganic phosphorus in the food (Noori, 2010). 60-80% of phosphorous will be absorbed in the gut. The absorption rate is greatest in the jejunum, although absorption occurs throughout the small intestine. Evidence has emerged that some forms of dietary phosphorous are less bioavailable. Although total phosphorous per g of protein is similar in animal products and plants, in plants most of the phosphorous (~75%) is in the form of phytate, which is poorly digested. Therefore, less phosphorous is absorbed from foods (Kemi, 2010). The results obtained in the present study in the proportion of phytate and non phytate phosphorus in the samples in mg/100g and percentage (Table 7).

4.7.1 Phytate phosphorus content

The mean phytate phosphorous content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 63.5-74.9mg/100g as shown in (Table 7). In the present study the phytate phosphorous ranged from 9.6 - 34 % for control and oven dried respectively. The phytate phosphorous content of control *injera* was significantly different from freeze dried sample which also significantly differed from sun dried and oven dried sample. The

low phytate phosphorous content of *injera* prepared from prefermented flour might be due to the less phytate content. The phytate phosphorous of the control sample has a slight variation with the report of (Urga, 1997) which was reported as 38.4, in a white *teff injera* in a dry weight bases. The variation might be due to the agronomical practices in the production or variety of *teff* used for analysis.

Table 7 Phytate and non phytate phosphorous composition of *teff injera* prepared from prefermented flour prepared with different drying methods

Drying Tech.	Phytate P ^a		Non phytate P ^b	
	mg/100g	%	mg/100g	%
Sun Dried	68.70±0.02 ^c	12.10	499.15±22.68 ^b	87.90
Oven Dried	63.47±0.43 ^d	9.62	596.33±11.75 ^a	90.38
Freeze Dried	74.92±0.90 ^b	14.76	432.93±10.48 ^c	85.25
Control	81.46±0.09 ^a	33.96	158.44±11.40 ^d	66.04

a= phytate phosphorous calculated by assuming phytate contains 28.18 % phosphorus (phytate * 0.28) b= non phytate phosphorous = total phosphorous - phytate phosphorous. Control represents the traditionally prepared *injera*.

4.7.2 Non phytate phosphorus content

The mean non phytate phosphorous content of *injera* which were prepared from instant prefermented flour using different drying methods ranged from 432.9mg/100g-596.3mg/100g (Table 7). In percent the non phytate phosphorous ranged from 85.3 % - 90.4%. The non phytate phosphorous content of control *injera* gave significantly (p<0.05) lower mean followed by freeze dried which also significantly differed from sun and oven dried and samples. The non phytate phosphorous of the control sample has a variation with the report of (Urga, 1997) which was reported as 277.2, in a white *teff injera* in dry weight bases. The variation might be due to the agronomical practices in the production or variety of *teff* used for analysis.

5 Conclusion and recommendation

5.1 Conclusion

The prefermented flour prepared was acidic in its properties. Therefore, it will have better shelf stability than unfermented flour. The freeze drying process takes longer time to dry the prefermented dough but with better quality flour. The oven drying took shorter time to dry followed by the sun drying.

In the use of prefermented flour, the preparation of injera was successful when it was baked using baking powder. The results in the sensory quality evaluation of *injera* showed that all the processing methods got acceptability score of greater than five in a nine point hedonic scale. From these results it can be concluded that there is a potential of preparing injera from prefermented flour. The freeze dried and control samples got similar acceptability in almost all attributes. Sun and oven dried has been scored with lesser scores in all attributes.

From the chemical composition analysis of injera prepared from prefermented instant flour indicated that nutritional composition has been improved. From minerals Fe, Zn and P; from proximate composition protein, ash and fiber and from were increased. The phytate content was decreased which will improve the bioavailability of minerals like iron, calcium, and zinc.

5.2 Recommendations

The following recommendations are made based on the study conducted and findings on the possibilities of preparing injera from prefermented instant flour:

- The results have shown that there is a possibility of industrializing *injera* preparation process using instant prefermented flour therefore entrepreneurs can take it as a business idea and apply.
- Other researchers should work on a design and operation of dehydration equipment that aims to minimize cost and undesirable changes in the drying process with special specification of the product.
- There should be a research on the possibility of enhancing the microorganisms inactivated in the drying process to be active and produce the desired carbon dioxide in the eye formation and to avoid use of baking powder.
- Other researchers should work on the suitable baking powder to be used in the process of instant *injera* preparation from prefermented flour with optimized speed of action and minimized after taste.
- Other researchers can isolate fast acting yeast strains from the common fermenter yeasts of *injera* to be used with the prefermented flour prepared.

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7 Appendixes

7.1. Sensory evaluation score card

Sensory Acceptability Test

The purpose of this sensory test is to check which processing treatment is more relevant in its acceptability by panelists.

Instructions

Please rinse your mouth with water before starting and between each tasting.

Taste the samples according to the numbers indicated and give value from 1-9 for each attribute based on the key given below.

If you have any question please ask the server.

Keys

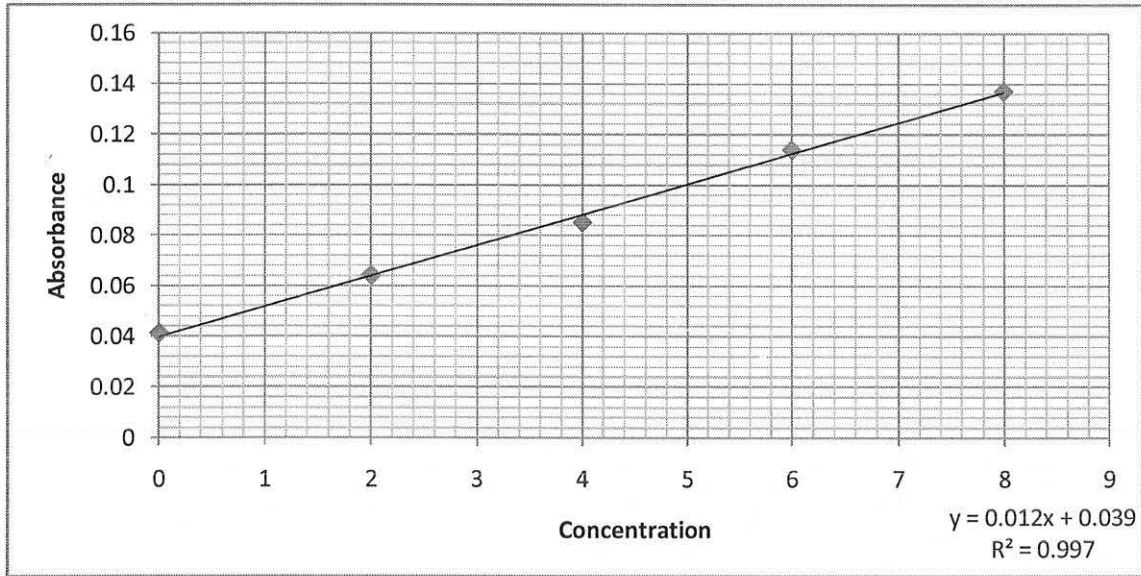
- | | |
|-----------------------------|--------------------|
| 1. Dislike extremely | 6. Like slightly |
| 2. Dislike very much | 7. Like moderately |
| 3. Dislike moderately | 8. Like very much |
| 4. Dislike slightly | 9. Like extremely |
| 5. Neither like nor dislike | |

Attributes	011	012	013	014
Fluffiness				
Taste				
Eye Size and distribution				
Top and bottom surface color				
Appearance				
Aroma				
Over all acceptability				

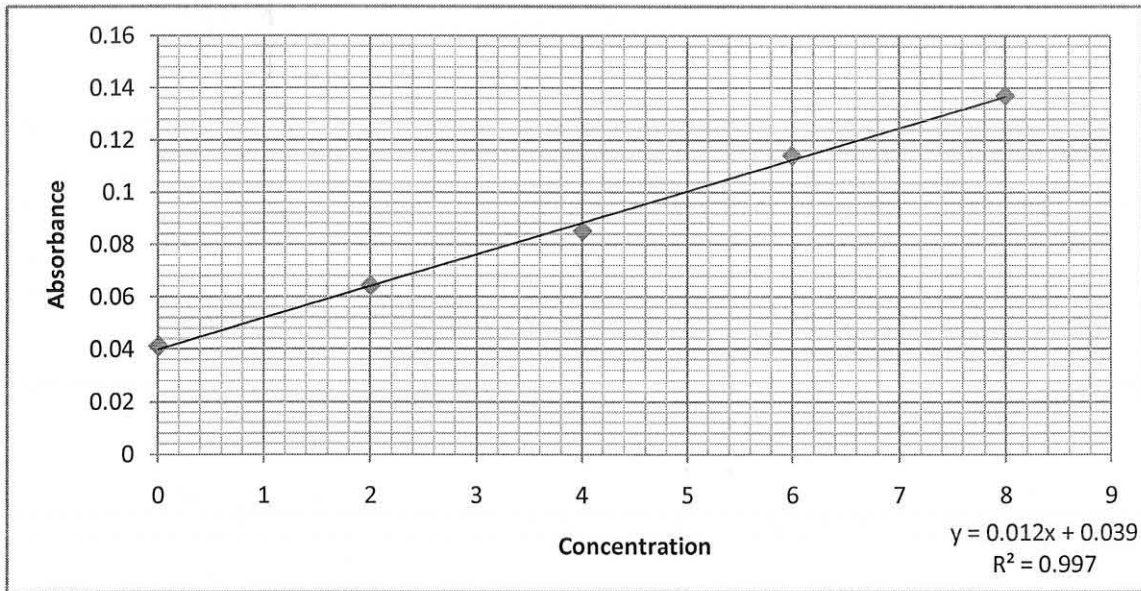
General Comments:

7.2 Calibration curves

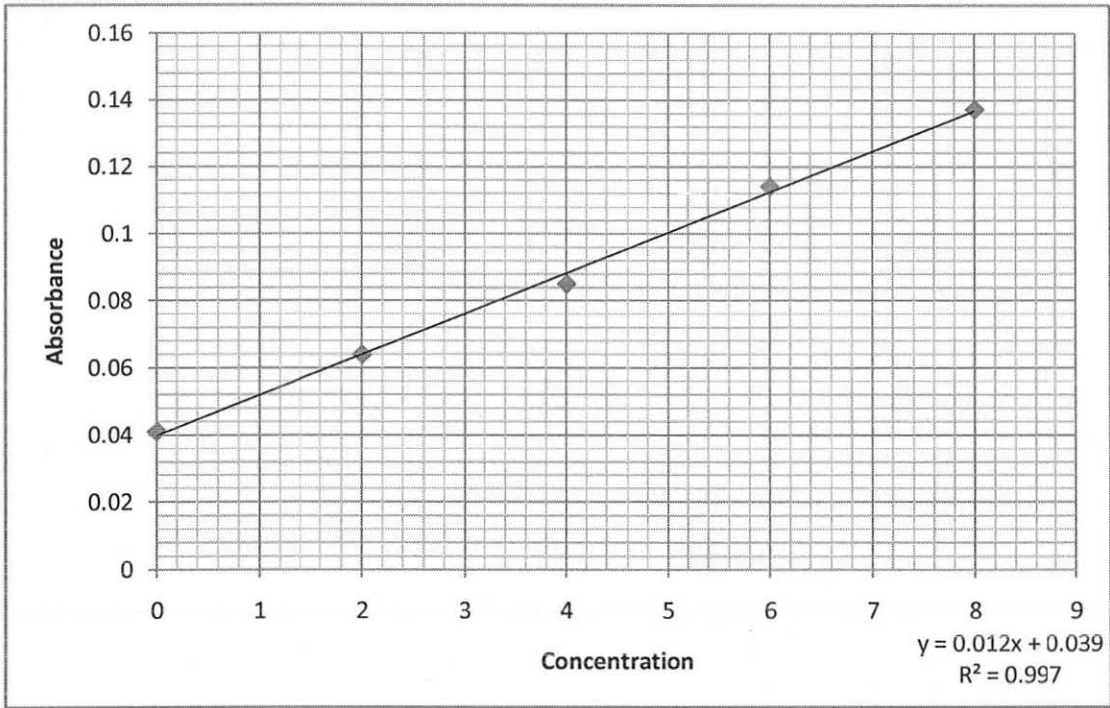
7.2.1 Phytate



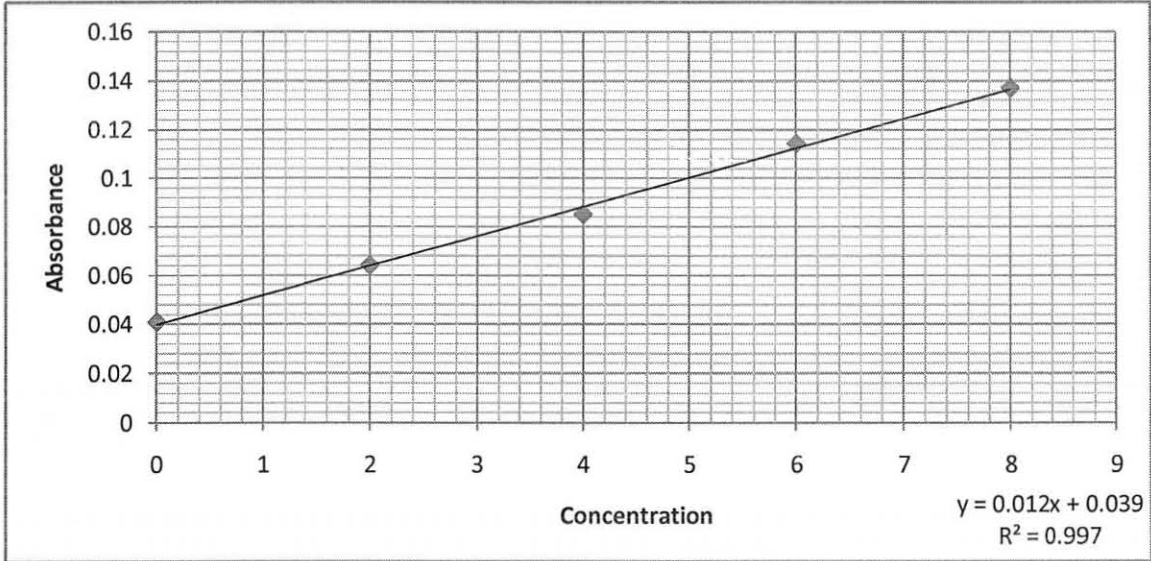
7.2.2 Tannin



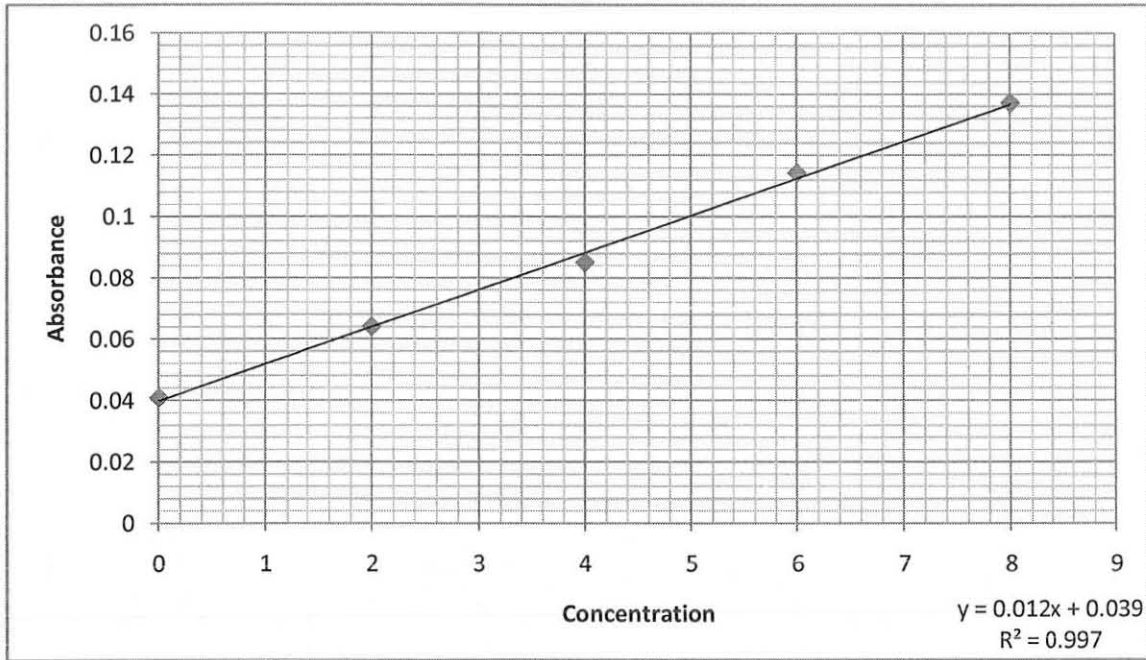
7.2.3 Iron



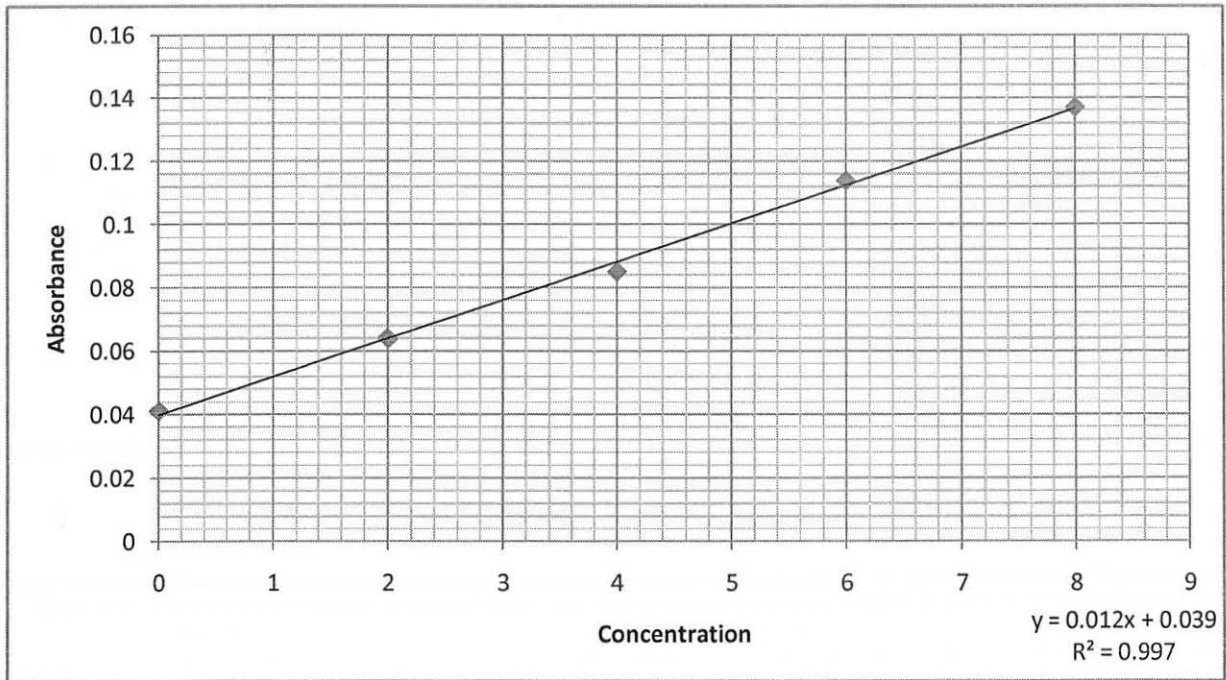
7.2.4 Calcium



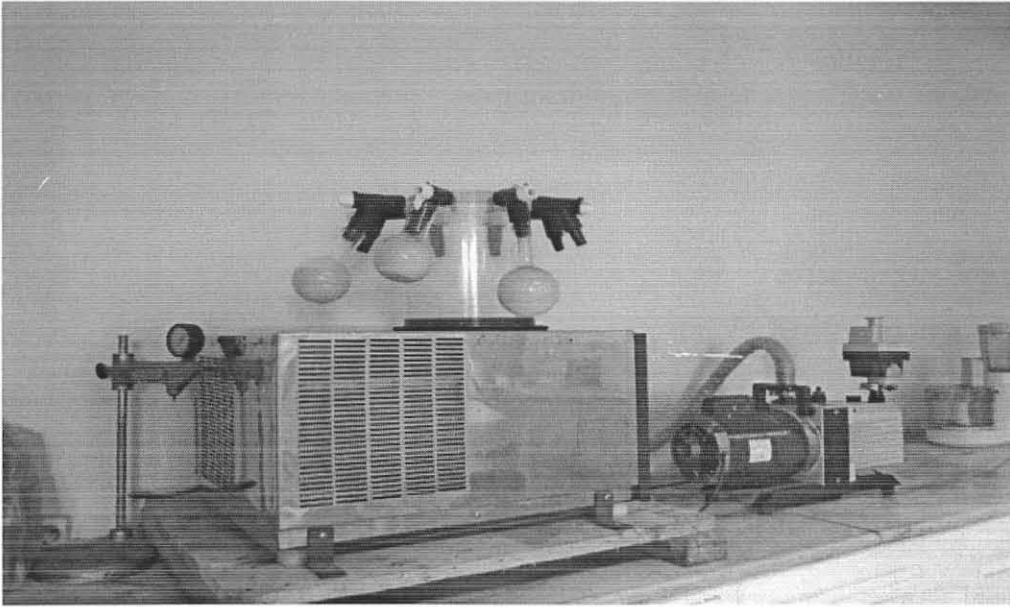
7.2.5 Zinc



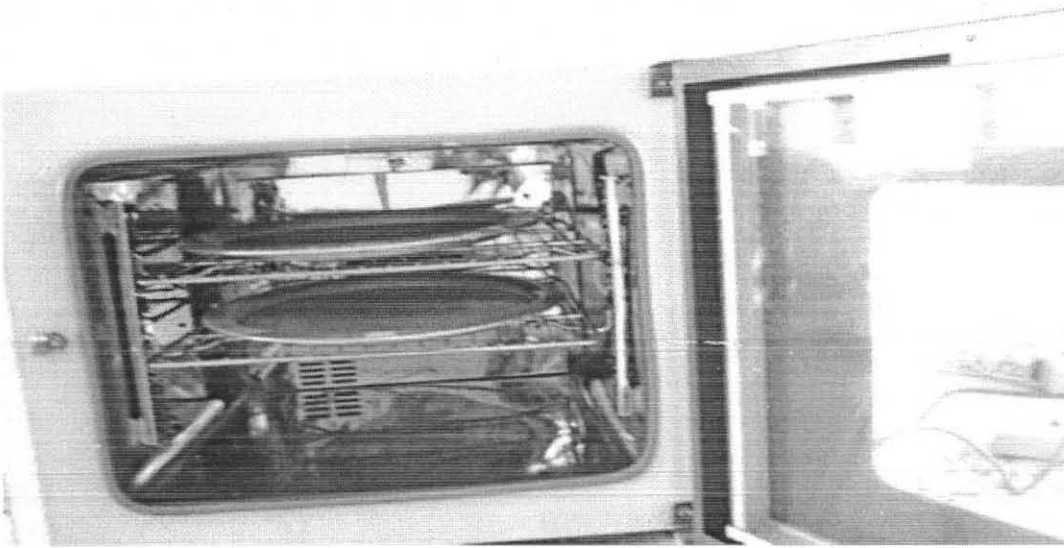
7.2.6 Phosphorous



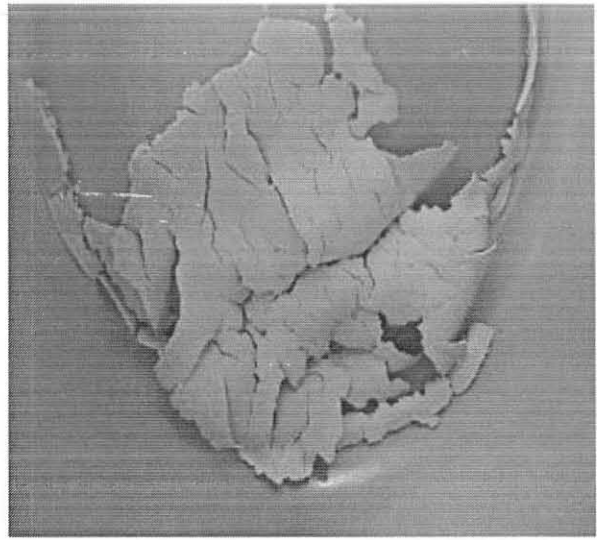
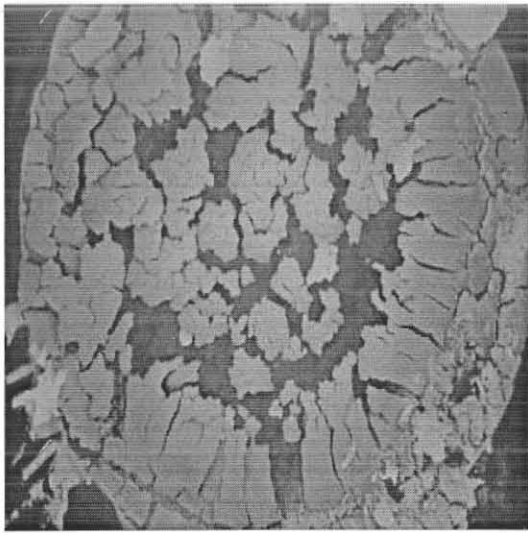
7.3 Equipments Used



Sample drying using freeze drier.



Sample drying using Oven drier.



Sample drying using sun drying

DECLARATION

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

Name: Hiwot Tadesse Abiyu

Signature: 

Date: 21/10/2004.

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

Name: Dr. Gulelat Desse

Signature _____

Date _____

Ato Ashagrie Zewdu

Signature _____

Date _____