ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Effect of formulated starter culture "*ersho*" on the biochemical profile of fermented *Teff dough* and *Injera* and studing there sensory quality and shelf life

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This is to certify that the thesis prepared by Amen Leye entitled: *Effect of formulated starter culture (ersho) on the biochemical profile of fermented Teff dough,Injera and studing there sensory quality and shelf life.* Submitted to the College of Natural and Computational Sciences of Addis Ababa University in partial fulfillment of the Requirement for the Degree of Master of Food Science and Nutrition fulfills with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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ABSTRACT

Fermentation is one of the most economical methods of producing and preserving foods. *Injera* is one of the most popular fermented foods among Ethiopians. However, occasional failures do occur in the fermentation that leads to inconsistencies in the quality of *Injera* and it is difficult to set certain standards for *starter cultures*. This study aimed to evaluate the effect of formulated starter cultures (*Aerobic mesophiles, molds, yeasts, LAB),*on biochemical profile of fermented *Teff* dough, sensory quality and shelf life of *Injera*. Experimentation and processing of data were performed on 2 *Teff* varieties (Kuncho and Bosete), 3 Treatments (3% traditional household starter culture (T1), 3% formulated starter culture in ambient condition (T2) and incubator condition(T3) and 7 Fermentation time intervals. Analytical methods were determined by using AOAC 2001.Shelf life of *injera* determined by using PDA plates prepared for yeast and mold determination and sensory anlysis were assessed using consumer-oriented sensory panel. Among the three treatments T3K and T3B had obtained least pH value (3.20, 3.24), highest average scoring of protein content (14.18%, 14.73%), Fe bioavailability (0.81, 0.81), phenolic content (348.19 mg GAE/g, 342.39 mg GAE/g) respectively. Besides, T2K, T2B had obtained lower pH value (3.43, 3.47), lower Fe bioavailability (0.82, 0.82), higher average scoring of protein content (13.40%, 13.93%), phenolic content (327.57mgGAE/g, 298.65 mgGAE/g) respectively. Among baked *Injera* samples T3K and T3B had obtained highest Fe bioavailability (0.64, 0.66), protein content (13.90%, 13.53%) phenolic content (425.10 mgGAE/g, 394.01 mgGAE/g) respectively. Besides T2K, T2B had obtained lower Fe bioavailability (0.69,0.76), higher protein (12.77%,12.57%), and total phenolic content (243.9 mgGAE/g ,360.10 mgGAE/g) respectively. Despite the T1K, T1B obtained lower Fe bioavailability (0.74, 0.73), least protein content (12.49%, 12.37%), and total phenolic content (170.71 mgGAE/g, 177.3 mgGAE/g) respectively. All the three treatments showed yeast and mold counts that ranged from ($3.1*10^3$-$4.1*10^4$ cfu/g) and the overall sensory acceptability ranged from (6.5-5.81) out of 7 . Based on the results obtained it could be concluded the formulated starter culture (T2,T3) showed significant effect ($p<0.05$) on physicochemical properties, mineral bioavailability, improvement of storage period of *Injera* extend up to 5 days.

Key words: *Injera, fermentation, Starter culture, Teff dough*

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ABBREVIATIONS

AAS - Atomic Absorption Spectroscopy

ANFs - Anti nutritional factors

ANOVA - Analysis of variance

AMC - Aerobic mesophilic

APVDFAC -Animal products, Veterinary Drug and Feed Quality Assessment Center

CFU - Colony forming unit

CHO - Carbohydrate

CRD - Completely randomized Design

DPPH - 1, 1 – Diphenyl - 2 – picrylhydrazy

DZARC - Debrezeit Agricultural Research Center

FAO - Food and Agriculture organization of the United Nations

FDA - Food and drug administration

FRAP - Ferric ion reducing antioxidant power

GRAS - Generally Regarded as Safe

LAB - Lactic acid bacteria

LSD - Least Significance Difference

NABRC - National Agricultural Biotechnology Research Center

NTC - Non-total coliform

SPSS - Statistical Package for Social Sciences

T1B - Bosete *Teff* fermented by traditional starter culture from household

T2B - Bosete *Teff* fermented by formulated starter culture in environment condition

T3K - Bosete *Teff* fermented by formulated starter culture in incubator condition

T1K - Kuncho *Teff* fermented by traditional starter culture from household

T2K - Kuncho *Teff* fermented by formulated starter culture in environmental condition

T3K - Kuncho *Teff* fermented by formulated starter culture in incubator condition

TA - Titratable acidity

TC - Total coliform

TPC - Total phenolic compounds

UV - Ultraviolet

VRBA - Violet red bile agar

VRBG - Violet red bile glucose

1. INTRODUCTION

1.1. Background

Fermentation is one of the most economical methods of producing and preserving foods. It provides a way to preserve food products, to improve organoleptic properties by producing different flavors of foods, improving the nutritive value of foods and to reducing toxic substances from foods (Thiele *et al*., 2002). Fermented plant food products are among the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for many people in the developing world (Blandino *et al*., 2003). In Ethiopia, there are several types of fermented food and beverage products and these include *Injera, kocho, bulla, tella, tej, ergo, ayib,* etc. These fermented foods and beverages products are consumed and being prepared from a wide range of raw materials using traditional techniques (Belay *et al*., 2018). *Injera* is soft fermented baked bread that is typically obtained after cereal flour has been fermented for 24 to 96 hours, depending on the ambient temperature (Askal and Kebede, 2013).

Injera is the most popular fermented food among Ethiopians. It is typical fermented pancake-like bread that is thin, flat, and many-eyed. Different cereal blends are used to make *Injera* depending on the agro-ecology of the region (Melaku, 2020). *Injera* is traditionally and preferentially produced from *Teff* flour (*Eragrostis tef* (Zucc.) in the northern Ethiopian highlands and around Addis Ababa, but wheat, barley, sorghum, millet, maize, and rice are all used elsewhere (Ashenafi, 2006). Good *Injera* is soft, fluffy and able to be rolled without cracking. Inclusion of ingredients such as *Teff* based flour for *Injera* preparation may be worth to improve the necessary characters. Various forms of *Injera* made from various grains have no significant variation in moisture, protein, carbohydrate, or phosphorus content (Zewdu and Abate, 2012). Like many other traditional fermented foods, fermentation in *Injera* formulation is originally spontaneous and dependent upon the load and flora of microorganisms naturally present in the flour, mixing water and airborne contaminants (Zewdu and Abate, 2012). In fermentation processes, various starter cultures and even back slopping are extensively used. A typical starter, is substrate-adapted that allows better control of a fermentation process and predictability of the products (Holzapfel, 1997).

Starter cultures facilitate control over the initial phase of a fermentation process. The prospect of applying starter cultures will become attractive as reducing of costs (e.g. energy), reduced fermentation times, reduced risk of spoilage, improved process control, sensory quality, safety attributes and reduced preparation procedures for the final product (Holzapfel,2002). Some LAB and yeast strains found in fermented foods are capable of decomposing anti-nutritional substances (Baye *et al*., 2014). As a result, using these microbes as starter cultures may improve the nutritional content of foods. Selected strains of starter culture may boost the overall benefits of fermentation such as better protein digestibility and micronutrient bioavailability (Holzapfel, 2002). Households are generally able to carry out consistently successful fermentations through practicing a system of back-slopping. Whereby a 'portion of liquid' known as '*ersho'* from a successful previous fermentation is used to inoculate freshly prepared dough (Mulatu, 2019).

The traditional method of *Injera* preparation varies from household to household and from region to region. As observed from baked *Injera,* about 9% of starch is known to be utilized by fermented microorganisms during dough fermentation (Tewodros *et al*., 2013). According to Mihrete (2019), the time necessary for *Injera* dough fermentation can be influenced by a variety of factors such as the microbial flora of '*ersho*' and flour, fermentation temperature, and the cleanliness of the container used.

Desiye *et al*. (2017) have collected 34 batter samples and reported the total aerobic mesophilic, lactic acid bacteria and yeast counts increased by about 3 log units until 48 hr. Fermentation, but the authors reported the decrease of *Enterobacteriaceae* below detectable levels after 18 hr. due to the low pH of the *Teff* batter. The fermentation process was characterized by the fall in pH from 5.0 to 4.2 and rise in the titratable acidity from 0.20 to 0.50% during 96 hr. of fermentation (Zewdie *et al*., 1997).

Yeasts that are responsible for *Injera* fermentation were identified as *Pichia fermentans, Pichia occidentalis, Candida humilis,* and *Kazachstania bulderi* in fermented *Injera* batter. This confirmed that the presence of different yeast species in the fermented mix and conformed the complex nature of dough fermentation (Tadesse *et al*., 2019).

Woldemariam *et al*., (2019) have examined *Teff* (40–100%), and barley (0–20%) flour combinations on proximate and mineral analyses and found that the nutritional and sensory adequacy of *Injera* increased. The predominant organisms identified were *Lactobacillus* and yeasts. Members of the Enterobacteriaceae initiate the fermentation during the first 18 hr. of fermentation and reduce the pH of batters to 5.8. In next stage, *Leuconostoc mesenteroides* and *Streptococcus faecalis* also observed and reduced pH to 4.7 (Gashe, 1985).

Lactic acid bacteria were responsible for the acidic character of the *Teff* dough fermentation (Guandalini, 2006). Although the major quality attribute of a good *Injera* is its slightly sour taste, which is due to the acidic nature of *Injera* (Zegeye, 1997). Its acceptance and palatability is also determined by its desired texture (stalling) and appearance. Unfortunately, the shelf life and those quality attributes of *Injera* are not stayed longer only 3 to 4 days (Zewdu and Abate, 2012). Molds play an essential role in spoilage of *Injera* (Hassen, *et al*., 2018). A*spergillus niger, Penicillium* sp. *and Rhizopus* sp. found to be responsible in *Injera* spoilage (Zewdu and Abate, 2012).

Very recently, Adeba (2021) has reported that consortia of microorganisms including *Enterobacteriaceae, aerobic mesophiles, mold, yeast, lactic acid bacteria (LAB), and coliform* involved in *Teff Injera* dough fermentation. The author further remarked that the formulation of these groups as starter cultures had significant effect on *Teff* dough fermentation and improves the quality of baked *Injera* product. Based on this finding, this study was aimed to evaluate the effect of this formulated starter culture ‘‘*ersho*’’ on the biochemical profile, nutritional quality of fermented *Teff* dough, sensory acceptability and shelf life of *Injera*.

1.2. Statement of the problem

In order to maintain and sustain African indigenous fermented foods and beverages, controlled fermentation and product quality characteristics are strongly recommended (Kabeir *et al*., 2004; Theodore *et al*., 2007 and Taiwo, 2009). Controlled fermentation uses pure or mixed starter cultures with an appropriate technology (Glover *et al*., 2009). Multifunctional starter cultures have the potential to improve the safety, shelf life, taste features, nutritional content, fermentation time, and even health-promoting properties of the fermented foods. Fermentation by microbial activities is a biotechnological method that historically arisen from the need to preserve food and the process improves the organoleptic properties of the fermented product (Ravyts *et al*., 2012).

In Ethiopia, fermented food preparation is predominantly a household phenomenon. Every household appears to process food starting from raw ingredients to the final products. Housewives bake *Injera* every 2 - 3 days, and know the usefulness of the starter culture. However, occasional failures do occur in the fermentation that leads to inconsistencies in quality of *Injera* (Askal and Kebede, 2013). *Injera* preparation is typically performed at the household level which often carried out using traditional practices. However, previous experiences indicated the presence of poor yield, sourness due to long hours of fermentation, insufficient nutritional value, and lack of consistencies. This could be contained by using biologically active components that can have a positive impact on quality, consistency and shelf life of the ready to consumed product (Ashenafi, 2002).

As long as *Injera* is stable food for Ethiopian people there are a lot of reviews done on formulating starter cultures for preparation of standardized and quality *Injera.* It is clear that while selecting starter cultures, features other than acid production by lactic acid bacteria or alcohol synthesis by yeasts should be considered (Holzapfel, 2002). Diverse yeast species are also responsible for *Injera* fermentation (Tadesse *et al*., 2019).

Research done on the microbial composition of 34 *Injera* batter samples identified 107 lactic acid bacteria strains (LAB) and 68 yeast strains had significant impact on the quality of *Injera* (Zewdie *et al*., 1997).

Since every household uses back slopping, the ready-to-consume *Injera* may not have the same taste, texture, appearance and overall acceptability, and difficult to set certain standard to *Injera* (Desiye & Abegaz, 2013).

It is clear that the process of baking *Injera* is absolutely traditional and has involved several steps with long process (Stewart and Getachew, 1962). In most household and *Injera* bakeries, the *Teff* batter, starter culture and back slopping processes have not been modified or improved for a better quality of *Injera* and reduction of fermentation time (ESA, 2013). The previous investigations have limitations of characterizing the microbial compositions of fermented *Teff* dough and some research findings target only LAB and yeast (Desiye & Abegaz, 2013).

Besides, a recent report shows that six different groups of microbes (*aerobic mesophiles, molds, yeasts, lactic acid bacteria (LAB), and nonpathogenic coliform*s) took part in fermentation of *Injera* dough by reducing the fermentation time and improving the quality of the baked *Injera* product. (Adeba, 2021). Therefore, the present study was initiated to determine the effect of the formulated starter culture on the physicochemical properties, proximate composition, mineral bioavailability, anti-nutritional factors, and antioxidant potential of fermented *Teff* doughs, sensory acceptability, and shelf life of ready-to-consume *Injera*.

1.3. Objectives

1.3.1. General Objective

To evaluate the effect of formulated starter cultures '*ersho*' on the biochemical profile of fermented *Teff* doughs and studing there sensory quality and shelf-life.

1.3.2. Specific objectives

- ✓ To evaluate the effect of formulated starter cultures '*ersho*' on the physicochemical properties, proximate composition, mineral bioavailability, anti-nutritional composition, antioxidant potential of fermented *Teff* doughs
- ✓ To evaluate the effect of formulated starter cultures '*ersho*' on the physicochemical properties proximate composition, mineral bioavailability, anti-nutritional composition, antioxidant potential of baked *Injera*
- ✓ To evaluate the effect of formulated starter culture '*ersho*' on the sensory quality of baked *Injera*
- ✓ To evaluate the effect of formulated starter culture *'ersho'* on the shelf life of baked *Injera*

2. LITERATURE REVIEW

2.1. Fermented foods

Fermented foods and drinks are tasty and nutrient-dense foods created from raw or heated raw materials (Adeba, 2021). Microorganisms contribute to the creation of distinctive qualities like taste, scent, visual appearance, texture, shelf life, and safety through their metabolic activities (Mainar *et al*., 2017). Through trial and error, traditional skills have been developed for controlling technical parameters during fermentation processes (Ray *et al*.,2017) Experience has also shown that back sloping, or the inoculation of raw materials with a residue from a previous batch, accelerates the initial phase of fermentation and results in the promotion of desirable changes during the fermentation process (Tadesse,2019).

Fermented foods can be found in almost all cuisines. Fermented foods have become in popularity in recent years in the west, owing to a variety of factors, including potential health benefits and an increased interest in gastrointestinal health. Fermented foods may benefit your health and help you fight disease in a variety of ways. To begin with, probiotic microorganisms such as LABs play a significant role in fermentation starting processes(Ray *et al* .,2017) The microbes, nutritional elements, and climatic circumstances all have a role in the fermentation process, resulting in hundreds of different types of fermented foods (Okoye,2017).Food fermentation has long been used as a technique of preservation since the production of antimicrobial metabolites (e.g., organic acids, ethanol, and bacteriocins) eliminating the likelihood of pathogenic microorganism contamination (Adesulu-Dahunsi,2020).

Fermentation of foods can be accomplished in two ways. To produce ethanol and carbon dioxide, yeasts consume carbohydrates such as fructose and glucose in the raw material. Many bacteria are toxic to ethanol (i.e., alcohol), but lactic acid bacteria are not bothered by it. In fact, certain lactic acid bacteria can survive higher quantities of ethanol than yeast. Other bacteria that cannot withstand acidic surroundings cannot develop because of the acid created by the bacterium. (Tilahun *et al* .,2018).

2.2. Ethiopian traditional fermented foods

Ethiopia is one of the countries that consume numerous fermented foods and beverages. *Kocho, Injera, awaze, tella, tej,* and *borde* are among them. Nonalcoholic fermented foods include *bukre, shamita, cheka, korefe, and merissa* and non-fermented foods include *kita, guenfo, atmit,* and *kinche* (Mulaw and Tesfaye, 2017). Lactic acid fermentation, fungal fermentation, and alkaline fermentation are the three types of traditional food fermentation. *Kocho* is a lactic acid fermented food in southern Ethiopia (Tafa and Asfaw,2020). Fermented food products provide diet food to consumers, practically utilized in developing countries. In addition, fermented foods are used as nutritious and balanced food, as well as anticancer, antiaging, anti-obesity, and ant constipation (Gitishree *et al*.,2020). Lactic acid bacteria and yeasts make up the majority of microorganisms found in fermented foods. Traditional fermented foods have existed in human diets since the beginning of time and are widely consumed (Anal, 2019). At home, you may make traditional fermented dishes and beverages in a variety of ways (Pumphrey *et al*., 2018). Fermented milk, fermented cereals, nonalcoholic beverages, fermented fruits and vegetables, and fermented meat are only a few examples. *Injera* is indeed a fermented food produced from a variety of cereals, including *sorghum, Teff, corn, wheat, and barley*, or a mixture of these cereals. Many Ethiopians prefer *Injera* made from *Teff* (*Eragrostis Teff*) to *Injera* made from other grains (Zhu,2018). *Injera* is a pancake-like meal made from cereal flour that has been fermented for 24 to 96 hours, carried out at ambient temperature (Mihrete and Bultosa,2017).

Major roles of fermentation to food products

Preservative Properties; - The preservative activity of LAB has been observed in some fermented products such as cereals. The lowering the pH to below 4 through acid production, inhibits the growth of pathogenic microorganisms (Ananou *et al*.,2007). LAB has antifungal activities. By doing this, the shelf life of fermented food is prolonged (Schnurer and Magnusson,2005)

Flavor Enhancement; -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 (Blandino *et al*.,2003).

Nutritional Quality; - Lactic acid bacteria have 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 22-25ºC (Mokoena *et al*.,2005). LAB fermentation also reduces the levels of anti-nutrients such as phytic acid and tannins in food leading to increased bioavailability of minerals such as iron, protein and simple sugars. The number of vitamins is also increased in the ferment (Santos *et al*.,2008).

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

Teff (*Eragrostis Teff* [Zucc.]) is a self-pollinated, annual, warm season grass that is used throughout the world as grain for human consumption and as forage for livestock. The amount of *Teff* produced in the world is increasing rapidly due to the plant ‘s popularity as an especial nutritious grain. *Teff* has been cultivated and utilized for human consumption mainly in Ethiopia since time in memorial (van Delden, 2011). However, currently with the understanding of the nutritional and health benefits of *Teff* grain, the crop is gaining popularity in the Western world menus and serious efforts are being undertaken to expand its cultivation notably in the Netherlands and United States of America and Spain (van Delden, 2011). *Teff* can grow in a wide range of agro-ecologies. It grows best between 1800 and 2100 meters, with an annual rainfall of 750-850 mm and a temperature range of 10-27^{0}C, yet it may grow in considerably wider ranges with rainfall of up to 1200mm. The growing period spans from 60 to 180 days (depending on variety and altitude), with 90 to 130 days being ideal (Ketema, 1997).

Teff is one of the world's most nutritious alternative grains. It contains calcium, fiber, protein, and antioxidants are all present. *Teff* is higher in nutrients and easier to digest than wheat, owing to its lack of gluten. It is composed of 11% protein, 80% complex carbohydrates, and 3% fat (Table 1). It is a rich source of essential amino acids, particularly lysine, which is frequently low in grain meals. (Doris Piccinin, 2010). *Teff* grain does not contain gluten and is an increasingly important dietary component for individuals who suffer from gluten intolerance. When grown as a grain it is normally ground into flour, which is used to make *Injera*, flat bread eaten with every meal in Ethiopia. It is also used as porridge, similar to cream of wheat or fermented and used to make an alcoholic beverage (Ketema,1997).

Table 1: Nutritional value of *Teff Injera* (ESA, ES 3786 ,2013)

S. No	Type of nutrients	Nutritional composition per 100 g of *Teff Injera*
1	Energy, *cal*	145.0 -155.9
2	Protein, *g*	3.0 -3.8
2.1	Gluten	ND*
3	Total carbohydrate, *g*	31.9 -34.0
3.1	Crude fiber	1.0 – 1.8
4	Fat,*g*	0.6 -0.7
5	Minerals (Total ash),*g*	0.7 – 1.7
5.1	Calcium (Ca), *mg*	50.0 – 68.0
5.2	Phosphorus(P), *mg*	100.0 -115.0
5.3	Iron(Fe),*mg*	7.0 – 14.7
5.4	Zinc(Zn),*mg*	1.5 -1.7

2.4. General description of *Injera*

Teff (Eragrostis tef Zucc.) and other grains, such as *sorghum, barley* are used to make *Injera*, a traditional meal. The grain is ground, combined with a batter, and fermented for several days with local unkown microbes. Cereal grain processing performance and nutritional value are mostly determined by their composition. Cereal grains are the world's primary source of carbohydrate and protein. They account for 70% of calorie consumption and 50% of protein consumption in human nutrition (Mulatu, 2019).Ethiopians' indisputable national cuisine is *Injera* (Blandino *et al*., 2003). *Teff* is the most commonly used grain in the production of *Injera*. A good *Injera* main quality feature is its somewhat sour flavor (Zegeye, 1997).

Injera is a fermented, pancake-like soft, circular flatbread similar to *chapatti* with small bubbly structures or eyes (honey comb-like holes) on its top surface which are produced due to the production and escape of CO_2 during fermentation and baking, respectively (Ashenafi ,2006).

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 (Gebrekidan and GebreHiwot ,1982). *Injera* has a very high nutritional value, as it is rich in calcium and iron. It is always consumed with stew (locally named in Amharic as ‘wot’) made from plant and animal products (Zewdu and Abate, 2012). *Injera* is also considered as good sources of energy, fiber, iron, calcium and vitamins although the fermentation process during preparation results in significant reduction of most of the nutrients found in the cereals flour. The major quality attribute of a good *Injera* is its slightly sour flavor (Mezemir, 2015).

2.5. *Injera* making procedures

The traditional method of preparing *Injera* varies from household to household and region to region. The major step on preparation of Injera from *Teff* is presented in (Fig.1). *Injera* is usually prepared in two stages of fermentation. The first takes 24-48 hours (depending on the desired sourness) from the time the flour is mixed with water and the ‘*ersho’*, back-slopping culture is added. Portion of the fermented dough is then cooked and added back to the fermented dough to begin the second fermentation. The mixture is reduced to a batter consistency and fermented for about 2-3 hours. After the gas bubbles have formed and subsided, the batter is diluted as needed, poured on a hot clay griddle, and baked while covered. The cooking gelatinizes the starch of the dough, the carbon dioxide produced during the fermentation trapped and leavens the Injera dough and as it escaped give a characteristic many-eyes to *Injera* (Steinkraus, 1983).

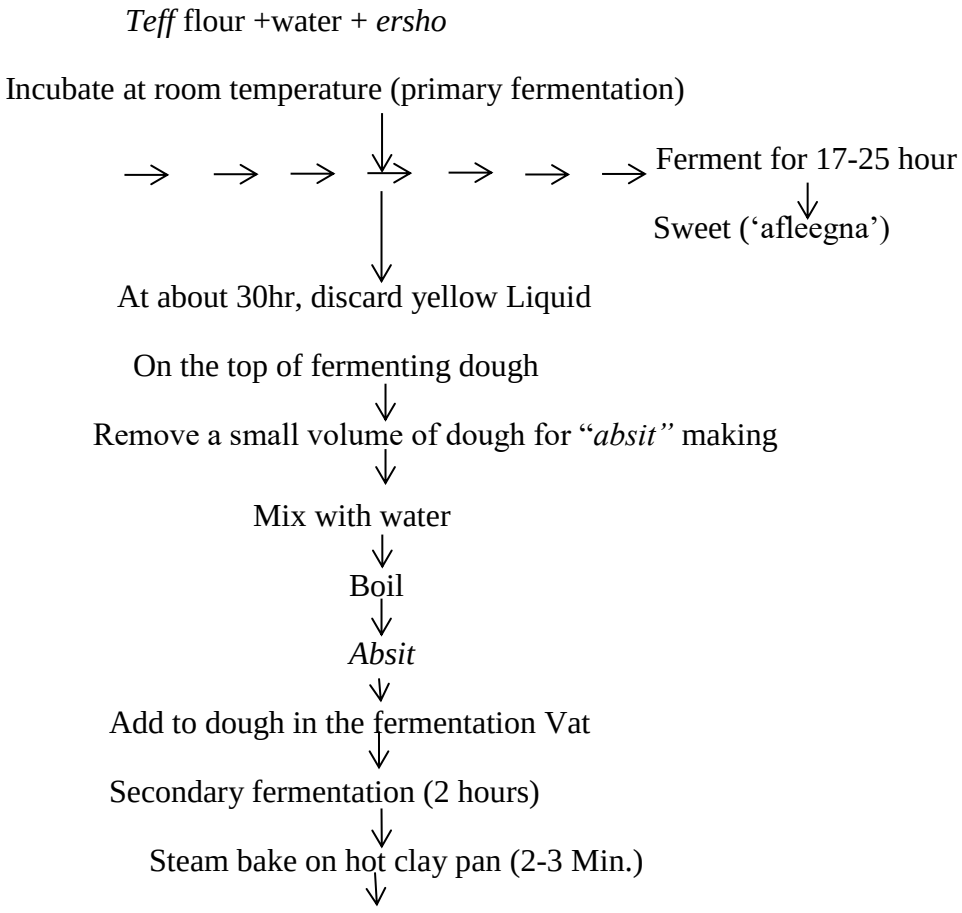


Figure 1: Steps on the preparation of Injera from *Teff (*Steinkraus 1983)

2.6. Fermentation process of *Injera*

Fermentation is the process by which a sugar is converted into an organic acid or alcohol by microbial action and enzymes. Fermentation occurs naturally in many foods, and humans have employed it to improve both food preservation and organoleptic characteristics since ancient times (Paulová, 2016). The preparation of *Teff Injera* consists of two stages of natural fermentation, depending on ambient temperatures. Temperature in the highlands of Ethiopia is generally between 17 and 25^{o} C. Primary fermentation of *Teff* flour usually lasts between 48 to 72 hours. It is initiated by the mixing of *Teff* flour, water and *ersho* (starter). After primary fermentation about 10% by weight of the fermented *Teff* is mixed with 3 parts water (1:3 ratio) and cooked for 2-3 minutes until the mixture boils and the starch gelatinizes (Boka *et al*., 2013).

This gelatinized mixture is referred to as *absit*. The *absit* is cooled and then added back to the fermented *Teff* batter and mixed thoroughly. This initiates the secondary fermentation of the batter which usually lasts between 30 minutes to 2 hours. After secondary fermentation, *Injera* is baked on a '*mitad*' or griddle for about 2-3 minutes at a temperature of 180 to 220 °C (Boka *et al*., 2013). An appropriate amount of flour is mixed with twice its weight of water. This is kneaded thoroughly to produce a thick paste. Inoculation is accomplished by adding some '*ersho'* (Attuquayefio,2014).

The fermentation process of *Teff Injera* initial 18 hours is characterized by vigorous evolution of gas and maximum dough-rising. 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 (Berhanu Abegaz ,1985). In relations to macro and micronutrient dynamics during the course of fermentation, some macro nutrients like protein decrease remarkable in the fermented *Teff* while minerals improved in their availability (Urga *et al*., 1997). In addition, fermentation plays a key role in reducing the inhibitors contents of *Teff* and increase the bioavailability of minerals (Umeta *et al*., 2005).

2.6.1. Factors affecting fermentation process

2.6.1.1. Fermentation time

Depending on the desired *Injera* flavor, Aflegna *Injera* is characterized by its thickness, sweet flavor, odor, and brownish red bottom and is made from batter that has only been fermented for 12 to 24 hours (Nout *et al.*,2007). Traditional *Injera* is fermented for 48 to 72 hours, and komtata *Injera* is created from an overly fermented batter. Over the course of the fermentation, the *Teff* settles on the bottom of the bohaka, leaving a yellowish or blackish liquid on top (Robert and Asnake ,1962). A portion of this '*ersho'* is saved for the next batch, and the rest is poured off. About 10% of the fermented paste is mixed with three parts of water and boiled (Sandor ,2021)

2.6.1.2. Aeration

Aeration is an important component that affects the fermentation process of many components. The presence of high aeration causes a decrease in the final ethanol yield (Andrew,2019). In the process, yeast creates acetic acid instead of alcohol, yeast destroys ethanol in the presence of air, or ethanol evaporates when gas exchange is not controlled (Andrew,2019). Aeration induces acetic acid production and if it affects the total ethanol yield, so limited air is a better fermentation condition to get a good product (Andrew, 2019).

2.6.1.3. The Microbiota of Injera sourdough

Different groups of LAB and yeasts are involved in *Injera* sourdough fermentation. All those identified microorganisms may be involved in the fermentation of Teff dough during *Injera* preparation. At species level, knowing the microorganisms found in *Teff* dough at species level is Important for other aspects like starter culture formulation to solve problems facing *Teff Injera* (Jula,2020)

2.6.1.4. Temperature

Temperature is an important factor for microbial growth; all microbes have a certain optimum range in which they can grow (Muhammed *et al.*,2018). Fermentation temperature has been found to impact the pH of spontaneous *Teff f*ermentations and quality of *Injera* (Penka and kaoyan,2020).Temperature control is critical in sourdough production as changes in fermentation temperature may cause variation in microflora of sourdough and thus variation in sourdough and final bread quality and flavor (Pasquale *et al.*,2019). The optimum temperature range for yeasts is 20-30°C.Lactobacillus species thrive at temperatures above 22 ^{0}C (Himoonga *et al*., 2021)

2.6.1.5. pH

Changes in the functional pH of the ethanol production process may utilize the main fermentation pathway. It is critical to maintain a pH range of 3.5 to 4.5, but if it exceeds this range, the production of by-products such as acetic and butyric acid may have eaten part of the substrates, lowering ethanol fermentation efficiency (Patrick *et al*., 2021).

2.6.2. Microorganisms involved in *Injera* dough fermentation

Major fermentation microbes include *lactic acid bacteria* and *yeasts* (Baye *et al*., 2013). Microorganisms have continuously been amazingly involved in *Injera* fermentation.

In general, a wide spectrum of microorganisms is involved during fermentation processes but a few types usually determine quality of the end product. (Tesfaye *et al*., 2018)

2.6.2.1. Yeasts

Yeasts and their metabolic products have been utilized in numerous food preparation and conservation around the world, basically for baking and brewing (Voidarou *et al*., 2021). *Candida humilis, Pichia spp., Kazachstania exigua, Wickerhamomyces anomalus, Candida famata and S. cerevisiae* are the most common yeasts strains, which are responsible for the fermentation of sourdough (Ahmed *et al*., 2013). The yeast species dominating the fermented dough are those that are able to adapt to the changing intrinsic conditions caused by physicochemical changes, due to microbial activity (Navarrete-Bolaños, 2012).

2.6.2.2. Lactic acid bacteria (LAB)

Among bacteria, *lactic Acid Bacteria* are one of the most dominant groups of organisms in *Teff* dough fermentation (Tadesse *et al*., 1999). LAB is used for the fermentation of food and feed products since ancient days and today their major applications are still in the food and feed industry as starter cultures (Ishola & Adebayo-tayo, 2012). LAB is the most important bacteria used in the fermentation industry in combination with yeast are commonly used to ferment cereal products such as dough (Muhialdin *et al*., 2011). LAB is a heterogeneous group of microorganisms with GRAS (Generally Recognized as Safe) status that have traditionally been associated with food fermentation (Giraffa, 2004). Lactic acid bacteria were responsible for the acidic character of the *Teff* dough fermentation. Fermentation has been demonstrated to be more effective in the removal of gram negative than the gram-positive bacteria, which are more resistant to fermentation processing (Guandalini,2006).

2.6.2.3. Aerobic mesophilic

Among complex groups of microorganisms involved in *Teff* dough fermentation, members of *Enterobacteriaceae* and *aerobic mesophilic* bacteria were active during the first 18-36 hr. of the fermentation and reduced the pH of the fermenting dough to 5.8 (Gashe,1985). Desiye *et al*., (2017) reported that number of *Entrobacteiaceae* and *Aerobic mesophilic* counts were isolated from fermented *Teff* dough.

According to various researchers, the microbial ecology of fermenting *Teff* dough was diverse, with *Enterobacteriaceae* and *aerobic mesophilic* bacteria among the bacteria are the microbes involved in *Teff* flour fermentation (Guesh and Anteneh ,2017).

2.6.2.4. The Newly Formulated Starter Culture

The formulations were designed with six treatment groups having 59 possible combinations. The best six selected microbial isolates where each isolate representing each respective group : Aerobic mesophilic (*Lactobacillus casei strain, 431)* , Molds *(Clasdosporum strain),* Yeasts (*Pichia, Y-62),* lactic acid bacteria (*Lactobacillus casei (LB26), Lactobacillus casei strain 431(LB12), Lactobacillus paracasei (LB11) sub.strain Paracasei)*Non- total coliform *(Acetobacter strain),*total coliform(*Entrobacter strain)).* The consortia of three LAB isolates were involved in the formulation. Before use, the LAB, AMC, NTC and coliform isolates were separately refreshed in their respective broth media (MRS and nutrient) and incubated at 35º C for 48 hr. Yeast and Molds were refreshed in YEPD broth and incubated at 28º C for 3 days. For the preparation of a given defined starter culture formulation, a separate pure culture of each isolate was separately centrifuged at 5000 g for 5 min at 4º C and washed three times using buffer. Then pellet was dissolved in 10 mL sterilized distilled water The 1, 2 and 3% each of standardized microbes were inoculated in duplicate to 50 g *Teff* flour mixed in 70 mL sterilized distilled water (Adeba ,2021).

2.7. Baking of *Injera*

At the end of the first stage of fermentation, the liquid layer is discarded. After the liquid layer is removed, about 10% of the fermenting dough is combined with three parts of water and then boiled for 2 to 5 minutes. The boiled content This is known as *'absit,*' a dough enhancer, and it is combined with the rest of the dough in the fermentation vat. This process indicates the start of the second stage of fermentation. The dough-rising and gas formation processes are accelerated by mixing the boiled dough with the rest of the dough in the vat. The end of fermentation is signaled by maximum dough-rising, which generally takes 30 minutes to 2 hours. This step initiates the 'secondary fermentation'(Mogessie, 1994). The role of *absit* in *Injera* is similar to that of hydrocolloids in gluten-free breads (Zannini *et al*.,2012). According to the findings, '*absit'* has a substantial impact on the physicochemical and sensory quality of *Injera*. At this point, the fermenting dough is thin enough to be poured onto a hot flat pan, is wiped locally as a '*mitad*,' for steam-baking into *Injera*. The *Injera* pan is made up of clay and is 45-60 cm in diameter. Before baking, the heated pan is wiped with a piece of cloth after greasing it with kale and cotton seeds (Mogessie, 1994). Pouring the dough starts at the outside edge of the pan and moves clockwise to the middle. Within seconds, bubbles begin to develop, and the pan is covered with a lid. Thus, *Injera* is baked on the bottom side while steam bakes the top side. (Mogessie, 1994). Heat is transmitted from the heated pan to the surface of the food material during the baking process, while moisture is moved from the interior to the surface of the product and eventually evaporates. As a result, as cooking progresses, changes in temperature and moisture conditions occur, resulting in the desirable characteristics (color, texture, and flavor) of the food (Getenet, 2011).

The total baking time for one *Injera* is 2 ½ - 3 ½ minutes. The baking pan surface temperature in the experiment was found out about 215 °C on the pan surface in order to make it possible to bake 'nice Injera'. The temperature in the middle of the *Injera* during the baking process would reach around 90 °C (Ashenafi 2006). The *Injera* is taken from the baking pan using a straw plate and left to cool. One *Teff Injera* weighs between 350 and 450 g. *Injera* can be stored for 3–4 days. A greater number of larger eyes are a highly desirable feature of a good Injera (Mogessie, 1994).

2.8. Physicochemical analysis of fermented *Teff* dough and *Injera*

2.8.1. pH

The growth and metabolism of microorganisms are influenced by pH (Bohraand Parihar, 2006). pH is an important factor affecting growth of microorganisms in food because it affects Microbial energy metabolism involving the buildup of hydrogen ion concentration gradients across membrane and microbial enzyme activity and stability of cellular macromolecules (Lucas, 2003).

Most foods are at least slightly acidic, since materials with an alkaline pH generally have a rather unpleasant taste. Some foods are characterized by inherent acidity; others owe their acidity or pH to the actions of certain microorganisms. The acidity of a product can have important implications for its microbial ecology and the rate and character of its spoilage (Adams and Moss, 2000).

It has been well established that most microorganisms grow best at pH values around 7.0 (6.6-7.5), whereas few grow below 4.0 (Jay, 2000). Bacteria tend to be more fastidious in their relationships to pH than molds and yeasts, with the pathogenic bacteria being the most fastidious (Ray & Bhunia, 2008). The majority of the bacteria isolated dough samples were capable of hydrolyzing starch, implying that an increase in reducing sugars within 48 hours of fermentation could be due to amylase activity originating from flour and microorganisms. The lactic acid bacteria precede the fermentation by lowering the pH to 4.0 (Berhanu Abegaz Gashe *et al*., 1982).

The pH of the *Teff* dough was reduced during *Teff Injera* fermentation from about pH 5.8 to pH 3.8. (Umeta and Faulks 1989). When the pH of fermenting *Teff* reached 5.0 - 5.5, the growth of *Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus, and Pseudomonas aeruginosa* was inhibited (Meaza Girma *et al*., 1989). The pH of *Injera* batter fermentation with LAB starters (*L. plantarum and L. fermentum*, as well as their co-culture *S. cerevisiae*) decreased pH from 6.35 to 4.5 within 18-24 hr. (Valjakka *et al*., 2003).

2.8.2. Titratable acidity

Titratable acidity is given as the amount organic acid of food sample and represents the total amount of hydrogen ions contained in the food. Even though the words are sometimes used interchangeably, titratable acidity differs from total acidity. (Zewdie *et al*., 1997). Titratable acidity is other predictor of acid's impact on flavor. The titratable acidity of Injera batter fermentation with LAB starters *L. plantarum and L. fermentum*, as well as their co-culture *S. cerevisiae*) increased titratable acidity from 0.33 to 0.95 % (lactic acid w/w) within 18 to 24 hr. (Valjakka *et al*., 2003)

2.9. Nutritional profile of fermented *Teff* dough and *Injera*

Fermentation leads to synthesis and availability of nutrients, reduction in ant nutritional factors and also leads to a general improvement in the shelf life, texture, taste and aroma of the final product (Blandino and Webb, 2003). According to Mehta *et al*. (2012), fermentation alters food shape, texture, and flavor, increases its nutritional value, and promotes safety. Because of a little grain with a larger proportion of bran versus endosperm and germ *Teff* has substantially higher crude fiber, total and soluble dietary fiber content than wheat, sorghum, rice, and maize (Bultosa,2007)). In contrast to most common cereals, the amount of uronic acid in *Teff* grain is higher (Umeta, 1986). The crude protein content of 13 *Teff* types ranged from 8.7 to 11.1%, with a mean of 10.4% (Bultosa, 2007). *Teff* flour was reported to have proximate composition of about 11% protein, 73% carbohydrate, 3% crude fiber, 2.5% fat and 2.8% ash (Bultosa and Taylor,2004). Fermented foods can have the added benefits of enhancing flavor, and improving the nutritional value This is due to growth and action of the bacteria during fermentation (Jeyaram *et al*.,2009). The long fermentation time was increased the moisture and protein content, on the other hand ash, fat, fiber, carbohydrate and energy was observed maximum in short fermentation time. (Mihrete and Bultosa, 2017). According a research report on 13 *Teff* varieties, the proximate compositions were in some ranges: moisture content 9.30 – 11.22%, protein 8.70 – 11.10% , ash 1.99 – 3.16% crude fat 2.00 – 3.00% ,crude fiber 2.60 – 3.80% (Geremew,2007).

2.10. Bioavailability of minerals in fermented *Teff* dough and *Injera*

Minerals are important for various physiological functions in the human body and cereal grains are important sources of minerals. *Teff (Eragrostis Teff)* has been designated as a super grain because it is a healthy alternative to conventional grains for achieving a gluten-free diet (Satheesh & Fanta,2018). Even though, there are high variability in the mineral content reported in different studies, *Teff* is richer in such minerals like Ca, Zn, Mg, Fe and Cu as compared to the contents found in other cereal grains compared to the other cereals. The contents of a range of minerals of *Teff* grain are given as calcium (180 mg/100 g), potassium (427 mg/100 g), sodium (12 mg/100 g), iron (7.63 mg/100 g), magnesium (184 mg/100 g), phosphorous (429 mg/100 g) and zinc (3.63 mg/100 g) on wet basis were reported (USDA, 2017). The mechanism by which phytate inhibits mineral absorption is based on the formation of insoluble phytate-mineral or peptide-mineral phytate complexes in the gastrointestinal tract (Weaver and Kannan 2002). Furthermore, phytates form complexes with endogenously secreted minerals such as zinc (Manary *et al*., 2002) and calcium (Morris and Ellis 1985), making these minerals unavailable for reabsorption into the body.

Ramachandran and Bolodia (1984) evaluated the effect of fermentation on the bioavailability of iron, zinc, and by dialysis of *Teff* batter and reported increases in dialyzable portions of iron from 9 to 24%, and zinc from 2 to 43%. Fermentation may increase mineral bioavailability is useful in countries where fermented foods like *Injera* are widely consumed (Mohammed *et al*., 2011). Traditional fermentation leads up to 49-66% of reduction in phytic acid. Studies indicated that adding 10 mg/100g phytic acids to 19 bread rolls decreased iron absorption by 20%, and that adding 20 mg/100g decreased iron absorption by 40% (Adeyeye *et al*.,2000).Absorption of iron from cereals can be increased by the degradation or removal of phytic acid with simple technologies like fermentation (Hurrell, *et al*., 2003).Fermentation plays a key role in reducing the phytic acid concentration of *Teff* and in increasing the bioavailability of minerals (Umeta *et al*., 2005).

2.11. Anti-nutrition factors composition in fermented *Teff* dough and *Injera*

Ant nutrients are chemicals which have been evolved by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value. Some of the ant nutrients have been shown to be evidently advantageous to human and animal health if consumed in appropriate amounts (Ugwu & Oranye, 2006). Phytate is ubiquitous among plant seeds and grains, comprising 0.5 to 5 percent (w/w) (Loewus, 2002). The major concern about the presence of phytate in the diet is its negative effect on the mineral uptake (Greiner & Konietzny, 2006). The daily intake of phytate can be as high as 4500 mg. Fermentation time had a significant reduction effect on the phytic acid content of the blend *Injera* (Urga *et al*.,1997).

Oxalate is a common and widespread component of most plant families (Liebman, 2002). While its levels in many plants are generally low, it is found in high concentrations in the leaves, and conus of plants consumed daily that are of concern. When oxalic acid is consumed, it irritates the lining of the gut and can prove fatal in large doses. Currently, patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50- 60 mg per day (Massey *et al*., 2001). Tannins are found almost in all plants all over the world(Anonymous, 1973). The ant nutritional factors of tannins depend upon their chemical structures and dosage and the total acceptable tannin daily intake for a man is 560 mg. Fermentation time had significant reduction effect on the reduce the anti-nutritional factors tannin (Mohammed *et al*., 2011).

In Ethiopia, traditionally, the fermentation process is used to make *Injera, a Teff* pan-cake used to eliminate ANFs (Satheesh &Fanta, 2018). Anti-nutritional factors in food reduce nutritional value of food, interfering with digestibility, and absorption of nutrients. *Teff* contains high amounts of phytate with a wide range of variability, probably due to differences in varieties and growing conditions. *Teff* phytate content is comparable to values reported for wholegrain cereals (Schlemmer *et al*., 2009). Phytate can be degraded by endogenous phytases which can be activated by food processing techniques like soaking, fermentation, and germination and to a lesser extent during cooking. (Baye *et al*., 2013, Baye *et al*., 2014).

2.12. Antioxidant properties of fermented *Teff* dough and *Injera*

Antioxidants are substances capable of inhibiting oxidation, reducing the concentration of free radicals in the body and preventing lipid peroxidation (Ozsoy *et al*., 2008). Appropriate diets that include fruits, vegetables, whole grains, and pseudo cereals may contribute to good health due to the rich source of anti-oxidants. (Calderelli *et al*., 2016).

2.12.1. Phenolic acid

Phenolic acids are considered to be major contributors to the antioxidant capacity of plants. These antioxidants also possess diverse biological activities, such as anti-inflammatory, ant atherosclerotic, and anti-carcinogenic activities. (Boka *et al*., 2013). Plants rich in phenolic are being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food (Saeed *et al*., 2012). Before and after fermentation, the contribution of soluble phenolic extracts to total phenolic content of *Teff Injera* ranged from 14 to 17 % and 17–32 %, respectively (Shumoy *et al*., 2017).Total phenolic content was higher in red *Teff* (11.47 mg/g) as compared to brown *Teff* (9.72 mg/g) and white *Teff* (8.28 mg/g) The results showed that the phenolic content of raw Teff varieties were significantly different ($P<0.05$).In a study conducted on flour of seven pure *Teff* varieties, TPC ranged from 263 to 448 mg gallic acid equivalents (GAE)/100 g dry matter (dm), of which the bound phenolic content contributed to more than 84% of TPC (Shumoy and Raes, 2016).

2.12.2. Antioxidant activity assays on fermented *Teff* dough and *Injera*

2.12.2.1. DPPH scavenging activity

DPPH (2,2'-diphenyl-1 - picrylhydrazyl) is a stable radical compound frequently used to examine free radical scavenging activity of natural compounds (Amarowicz *et al*., 2004). This purple color generally disappears when an antioxidant is present in the medium/ low. Thus, antioxidant molecules can quench DPPH free radicals and convert them to a colorless product resulting in a decrease in absorbance at 517 nm (Gursoy *et al*., 2010; Woldegiorgis *et al*., 2014). Therefore, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract.

2.12.2.2. Ferric reducing power (FRAP)

Ferric reducing power is a novel ant oxidation defense mechanism that affects the property of electron transfer ability (Suttirak and Manurakchinakorn, 2014). The Fe^{3+}–Fe^{2+} reducing power of the extract may serve as a significant indicator of its potential antioxidant activity (Dastmalchi *et al*., 2007). The yellow color of the test solution change to various green and blue shade depending on the reducing power of each compound (Ferreira *et al*., 2007).The amount of Fe2+ can be monitored and determined by measuring the generation of Perl's Prussian blue at 700 nm and a higher absorbance indicates higher activity (Zarena & Sankar 2009).The higher ferric reducing activities can be attributed to higher amounts of polyphenolics and the reducing capacity of a compound may reflect its antioxidant potential (Lee *et al*., 2007). The FRAP of bound phenolic extracts significantly differed ($p < 0.05$) within each variety and among varieties at each fermentation time. FRAP of the bound extracts showed an increase of 30–40% after fermentation. The values of total FRAP of the combined soluble and bound phenolic extracts of the *Injera*. Noticeably, increased following fermentation of the dough from 0–120 hr. (Apak *et al*., 2016). The increase of FRAP in both the soluble and bound extracts was seen during the first 24 h of fermentation and remained relatively constant afterwards (Apak *et al*., 2016).

2.13. Shelf life of *Injera*

Food spoilage microorganisms are those that grow in food and produce undesirable flavor (odor), texture, and appearance, rendering the food unfit for human consumption. Mold spoilage is a serious problem among microbial spoilage that affects the shelf life of *Injera*, the staple Ethiopian fermented bread. *Aspergillus niger, Penicillium sp. and Rhizopus sp.* were found to be responsible for *Injera* spoilage (Zewdu and Abate, 2012). The shelf-life of a food is the period for which it remains safe and suitable for consumption. (Zewdu and Abate, 2012). Molds and yeasts are able to grow at lower pH than do bacteria (Ray and Bhunia, 2008). This indicates that molds are the responsible microbes for the spoilage of *Injera*. Even though molds take the responsibility for spoilage of *Injera*, some bacteria may be in charge under favorable conditions, such as high relative humidity, moisture content and optimum temperature (Jay, 2000).

2.14. Sensory evaluation of *Injera*

The quality of *Injera* is influenced to a large extent by the fermentation process and the length of time for fermentation. In *Teff*, the primary agent of fermentation of the *Injera* dough has been identified as the yeast *Candida guilliermondii ,Langeron and Guerra* (Stewart and Getachew 1962). Characteristics of *Injera* are directly related to its color, appearance, texture and taste. *Injera* may have a white or red color based on the variety of *Teff* grain used such as nech (white), kay (red), and sergegna (mixed) (Fellows, 1997).

The elastic texture and pores (referred to as 'eyes') formed on the surface of *Injera* are important quality attributes (Yetneberk *et al*., 2004). Although, good quality *Injera* has a mildly sour taste due to the use of sourdough as a starter for fermentation. Quality characteristics of Injera are directly related to its appearance, texture and taste (Bamforth, 2008).

According to Gebrekidan and Gebre Hiwot (1982), a normal and typical *Injera* is round, soft, spongy and resilient, about 6 mm thick and ~60 cm in diameter with uniformLy spaced honeycomb-like ''eyes'' on the top. A good Injera is also spongy and can be folded without cracking. The color of *Injera* could generally be whitish, cream, reddish brown, or brown depending on the color of the *Teff* flour used (Gebrekidan and Gebre Hiwot ,1982)

In taste, good *Injera* must be slightly sour to have the desired taste combination with the spicy wot. *Injera* made from dough that has not been sufficiently fermented has sweetish taste and is not considered good for eating with wot. This type of *Injera* is called aflegna. Texture is the overall experience of how a substance feels in the hand and mouth. It contributes to the overall eating experience and can impact flavor release of a food product. *Injera* is used as an eating utensil and this makes its texture an important quality attributes. (Gebrekidan and Gebre Hiwot ,1982). In order to determine consumer acceptability of product a sensory evaluation conduct with the parameters tested for are flavor, aroma, texture, color, and *Injera* appearance. (Stone and Sidel ,1985).

3. MATERIALS AND METHODS

3.1. Raw materials collection

The raw materials Kuncho –white *Teff* (DZ-Cr-387), and Bosete –white *Teff* (DZ-02-161) grain varieties were collected from Debrezeit Agricultural Research Center (DZARC). The newly formulated starter culture isolates were collected from National Agricultural Biotechnology Research Center (NABRC). As a part of *Injera* Project, these microbial consortia were isolated, characterized, formulated and maintained at NABRC. The traditional starter culture '*Ersho*' (source of yeast and other microorganisms) collected from a household. All the collected raw materials and the microbial isolates (starter cultures) were stored carefully until the appropriate and respective laboratory analyses were conducted.

3.2. Study setting

The study was laboratory-based experiments. The experiments were conducted on fermented of *Teff* doughs and ready-to-consume *Injera* samples. The experiments were conducted at Center For Food Science and Nutrition laboratory, Addis Ababa University and Animal products, Veterinary Drug and Feed Quality Assessment Center (APVDFAC). The prepared samples were kept in safe place until analysis takes place. A total of 42 sample in duplicate (84 samples) were tested from fermented *Teff* dough samples for biochemical profile laboratory analysis and total of 6 samples were tasted from baked *Injera* for biochemical profile, shelf life study, sensory evaluation of baked *Injera* samples.

3.3. Frame Work of the Experiment

The overall experimental procedures of the Thesis work are presented in (Fig. 2)

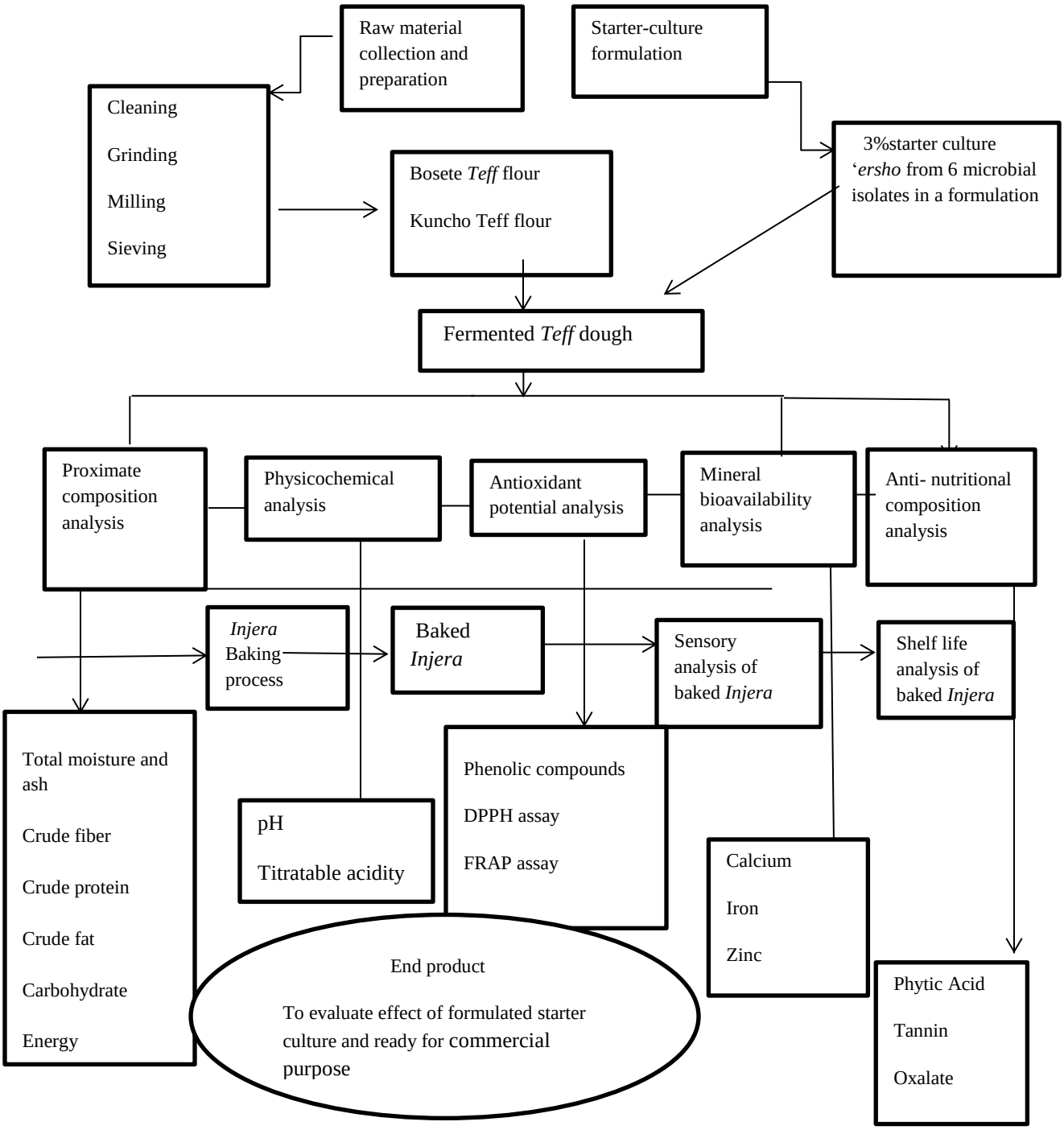


Figure 2: Experimental design

3.4. Processing methods

3.4.1. Flour preparation

Each *Teff grain*s was cleaned manually by sifting and winnowing before milling to remove the damaged grains, stones, dusts, light materials, glumes and stalks and other extraneous materials. Kuncho –white *Teff* (DZ-Cr-387), and Bosete –white *Teff* (DZ-02-161) samples were milled into fine whole flour using disk attrition mill with two disks, traditionally used in the cottage *Teff* grain milling house.

3.4.2. Starter culture formulation

In this study, the starter culture consists of six microbial groups*: aerobic mesophiles, molds, yeasts, lactic acid bacteria (LAB),* The microbial isolates (Fig.3a) in a starter culture formulation were separately refreshed in their respective broth media by incubating the bacteria at 35ºC and the fungal isolates at 25ºC for 48 hr. and 72 hr. respectively (Fig.3b). For the preparation of a given defined starter culture formulation, a separate pure culture growth of each isolate was separately centrifuged at 5000 g for 5 min at 4ºC and washed three times. Then pellet was dissolved in 10 mL sterilized distilled water. The *Teff* flour was sterilized using steam sterilization with lid tighten bowel (plastic jar) at 121^{o}C for 20 min. A 3% of formulated starter culture was added separately in duplicate to the mixed flour and sterilized water was added to mix thoroughly until homogenous slurry was obtained in 6 plastic jars.

a

b.

Figure 3: Formulation of starter culture isolates

3.4.3. Preparation of *Teff* dough

Dough was prepared; -

1) In the same way as done traditionally in every household;

2) By adding the formulated starter culture instead of adding traditional '*ersho'.*

Both Bosete *Teff* and Kuncho *Teff* flour (B, K) were used for each of the three treatments (T1, T2, T3). The resultant dough from each treatment was allowed to ferment for a time interval of 0 hr., 12 hr., 24 hr., 36hr., 48hr., 60hr. and 72 hr. (Table 2).

- Treatment 1 - *Teff* flour + 3% Traditional fermented starter culture (*ersho*) from household (T1B, T1K)
- Treatment 2 - *Teff* flour + 3% Formulated starter culture "*ersho*" in normal Ambient T^{o}condition (T2B, T2K)
- Treatment 3 - *Teff* flour + 3% Formulated starter culture *"ersho"* in Incubator (T3B, T3K) (Fig.4b),

Table 2: Series of working standards of treatments and *Teff* varieties

Treatment/Starter Culture (3%)	*Teff* varieties	Fermentation time (for each treatments)
T1 - (Traditional household Starter culture)	B - (Bosete –white *Teff* (DZ-02-161)	0HR 12HR 24HR 36HR 48HR 60HR 72HR
T2 - (Formulated *"ersho "*in Environmental condition	B - (Bosete –white *Teff* (DZ-02-161)	
T3 - (Formulated "ersho" in Incubator Condition)	B - (Bosete –white *Teff* (DZ-02-161	
T1 - (Traditional household Starter culture)	K – (Kuncho –white *Teff (*DZ-Cr-387)	0HR 12HR 24HR 36HR 48HR 60HR 72HR
T2 - (Formulated *"ersho "*in Environmental condition)	K- (Kuncho –white *Teff* (DZ-Cr-387)	
T3 - (Formulated "*ersho*" in Incubator Condition	K-(Kuncho –white Teff (DZ-Cr-387)	

A 1kg from Kuncho –white *Teff* (DZ-Cr-387), and Bosete –white *Teff* (DZ-02-161) flours was mixed with sterile municipal water in ratio of 1:2 (w/w) as was previously prepared (Zewdu and Abate,2012). A 3% of starter culture '*ersho*' collected from a household and the formulated starter culture was added in different sic plastic jars. (Fig.4A) The plastic jars were used for mixing their contents. After the primary fermentation of each treatment, the supernatant liquid which was slightly yellowish watery content on the surface of fermented batter was decanted (Fig.4c). For *absit* preparation, a separate content from each treatment of 200 mL of the fermented mixture was taken aseptically from the container into the saucepan and mixed with 400 mL of water and boiled (traditionally known as *absit*) on a hot plate with a continues stirring for about 5 min. The cooled separate *absit* (below 50°C) from each treatment was added to the main respective part of fermented batter and thoroughly mixed with stirring as indicated in Zewdu and Abate (2012). Intense fermentation for 2 hrs with a noisy air bubbles indicates the second phases of fermentation (Desiye and Abegaz, 2013).

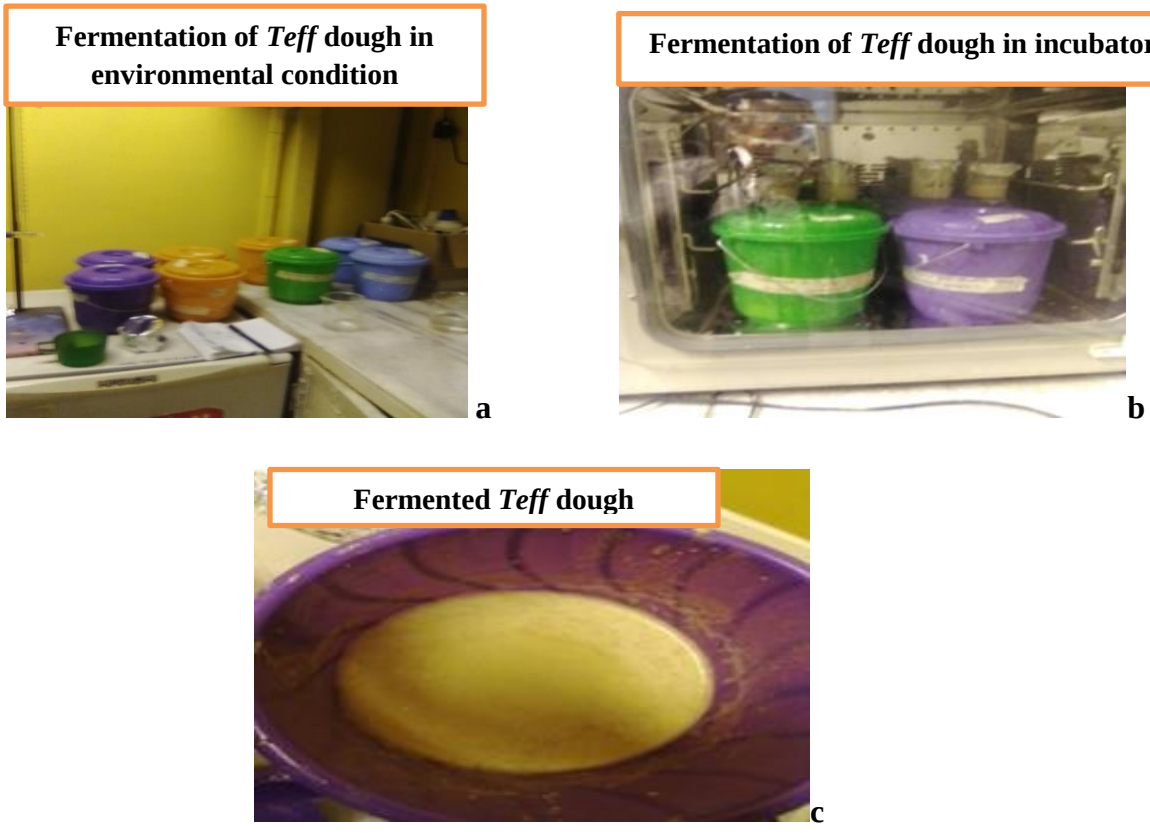


Figure 4: Fermented *Teff* dough preparation

3.4.4. Preparation of *Injera*

Fermented *Teff* batter from each separate treatment was baked by withdrawing 500 mL of fermented dough sample with sterile plastic beaker and poured onto the hot clay electric plate in a circular motion (Fig.5a). The baking temperature was between 190°C to 210°C controlled by checking using thermometer. After 2.5 -3 minutes baking, *Injera* was removed from the hot clay electrical plate (*mitad*) (Fig.5b) as indicated in Zewdu and Abate (2012). The *Injera* was baked from each of the three treatments a when the optimum fermentation time reached at 24 hr. for formulated starter culture in incubator condition (T3B, T3K), in environmental condition (T2B, T2K) at 48hrs and for traditional household starter culture (T1B, T1K) at 72hr. respectively. The baked *Injera* was removed from the plate and cooled to room temperature in a container (*mesobe)* (Fig.5c and Fig.5d) for further analysis.

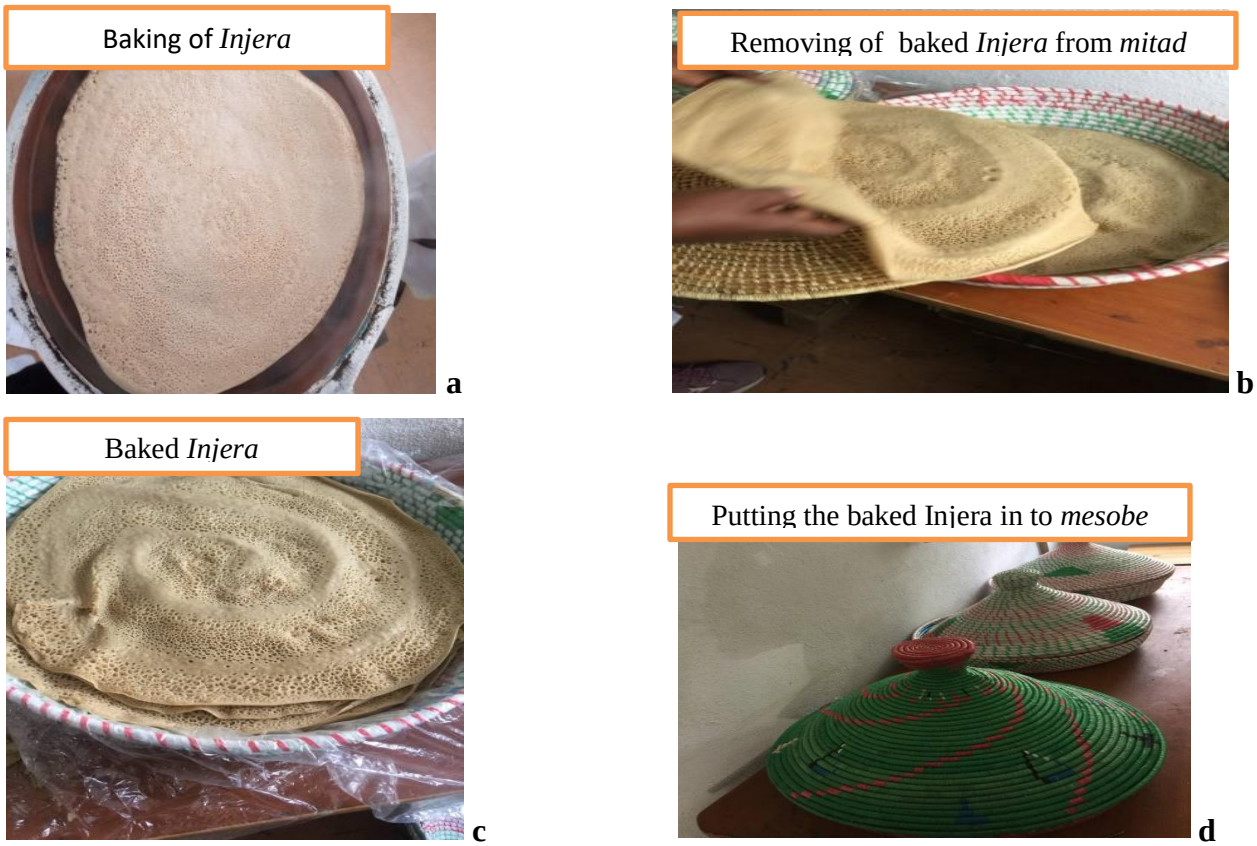


Figure 5:Preparation of *Injera*

3.5. Analytical methods

3.5.1. Physicochemical properties analysis of fermenting *Teff* dough

3.5.1.1. Determination of pH

The pH values were determined using AOAC, 981; 12 2001 official method using a digital pH meter (pH- 013 High Accuracy Portable pH Meter. The fermented *Teff* batter pH was determined directly using a glass electrode attached to a pH meter. The *Injera* samples (10 g) were mixed with 100 mL of distilled water, and the pH of the supernatant was immediately measured after decanting into a 250mL beaker.

3.5.1.2. Titratable acidity analysis

A modified method from (Drago, 1973) and AOAC 942; 15.2001was used to determine the total titratable acidity of the *Teff* dough samples. A beaker containing 10 g of the fermented *Teff* batter sample and 100 mL of distilled water. To behave as a control, the water used for dilution was first titrated before the sample was titrated. To a water extract of the sample, three drops of 1% alcoholic phenolphthalein indicator were applied. The dispersion was then titrated to the phenolphthalein endpoint by using a standard base (0.1N NaOH). The percentage of lactic acid consumed by a specific amount of 0.1 N NaOH was provided as the test result. When the white dispersion transitioned from a bright white solution to a turbid one that was faintly pink in color, the titration was complete. Finally, the amount of lactic acid in the sample was calculated using the formula (1 mL of 0.1 NNaOH = 0.009008 mg of $C_3H_6O_3$).

3.5.2. Proximate composition of fermenting *Teff* dough

3.5.2.1. Determination of Moisture Content

The moisture content of the fermented *Teff* dough samples was determined according to approved AOAC (2000) method 925.09. Briefly, a clean empty aluminum dishes and its lids were dried in drying oven (DHG- 9055A) at 100 ºC for 1 hour and cooled in a desiccator (CSN-SIMAX) with fresh granular silica gel desiccants for about 30 minutes and weighed. The prepared samples were mixed thoroughly and about 5.0 g of samples were weighed.

The dishes and their contents were placed in the drying oven and dried for 3 hr. at 105 ^{o}CAfter drying, the samples were cooled in desiccators for 30 min and reweighed until a constant weight is achieved. The amount of water lost from the sample was considered to be directly proportional to the loss of weight due to drying of the sample. The practice was repeated till the weight does not fluctuate. The percent moisture content was calculated by dividing the weight loss by the original weight of the sample as follows:

$$\%\text{Moisture}=\frac{(W2-W3}{W2-W1} *100$$

Where: W1= mass of the crucible, W2 = sample mass with the crucible, W3 = final mass of sample with the crucible

3.5.2.2. Determination of total ash content

The ash content of the fermented *Teff* dough samples was determined by incineration at 550 °C in a muffle furnace (Gallenkamp, size 3) until the ash is formed using the Official Method of the Association of Official Analytical Chemists (AOAC, 2001) with minor modifications. Cleaned, and dried porcelain crucibles were maintained for 30 minutes at 550 °C in a muffle furnace and cooled for about 20 minutes at room temperature in a desiccator (with granular silica gel); each crucible was weighed to the nearest gram (M1). 2 g of flour samples were weighed in each crucible (M2). To remove the water, the crucibles were first placed on a hot plate beneath a fume hood. The temperature was then steadily raised until the samples were thoroughly charred and no longer smoked. The crucibles holding samples were kept in a muffle furnace at 550 °C for four hours, then withdrawn and placed in a desiccator for 20 min to cool in order to weight and record the crucibles (M3).

The total ash was determined using the following equation: -

$$\%\ \text{Ash}=\frac{M3-M1}{M2-M1} * 100$$

Where: M1= mass of the crucible, M2 = sample mass with the crucible, M3 = final mass of sample with the crucible.

3.5.2.3. Determination of crude protein

The protein content of the fermenting *Teff* dough samples was determined according to approved AOAC (2000) method 979.09. About 0.5 g of samples were taken in a Tecator tube and 6 mL of acid mixture of concentrated orthophosphoric acid and concentrated sulfuric acid (5 parts of concentrated orthophosphoric acid and 100 parts of concentrated sulfuric acid) was added and mixed thoroughly and then, 3.5 mL of 30% hydrogen peroxide was added step by step. As soon as the violent reaction had ceased, the tubes were shaken for 3 min and placed back into the rack. A 3.0g of the catalyst mixture (ground 0.5 g of copper sulfate with 100 g of potassium sulfate) was added to each tube and allowed to stand for about 10 min before digestion.

The mixture was digested in the digester stove (HYP-1008 eight holes) at 370 ^{o}C for 4 hr. The digestion was continued for about 1 hr. until a clear solution was obtained. The tubes in the rack were transferred into the fume hood for cooling and 15 mL of distilled water was added to dissolve the precipitate and to avoid further precipitation of sulfate in the solution.

A 250 mL conical flask containing 25 mL of the boric acid indicator solution was placed under the condenser of the distiller (KDN-102F, nitrogen analyzer distillation device) with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5 mL distiller water and the rinses were added to the solution.

About 25 mL of 40% sodium hydroxide solution was added to the compartment and washed down with a small amount of water and the steam switched on. A 100 mL solution of the sample was distilled and then the receiver was lowered so that the tip of the condenser is above the surface of the distillate. The distillation was continued until a total volume of 150 mL is collected.

The tip was rinsed with a 3 mL of distilled water before the receiver was removed. The distillated solution was titrated with 0.1N hydrochloric acid to a reddish color and the amount of hydrochloric acid was recorded.

$$\% \text{ Nitrogen } \frac{(\mathrm{V}-\mathrm{B})\mathrm{x}\ \mathrm{N}\ \mathrm{x}\ 14*100}{1000*W0}$$

Where: V = the volume of HCL in L consumed to the endpoint of titration B = the volume of HCL taken in blank titration

N = Normality of HCL (0.1N),

WO = Sample weight on dry matter basis, while the molecular weight of nitrogen is14. Using an appropriate conversion factor, the percent of nitrogen is changed to the percent of protein as follows: Protein (%) = 6.25 * % Nitrogen

3.6.2.4. Crude fat determination

The crude fat content of the fermented *Teff* dough samples was determined according to approved AOAC (2000) method 920.39. the cleaned extraction flasks with boiling chips were dried in drying oven (DHG-9055A) at 90 ^{o}C for 1 hr., cooled in desiccators for 30 min and then weighed. The bottom of the extraction thimble was covered with about 2 cm layer of fat-free cotton. About 5 g of samples were added into the extraction thimbles and then covered with about 2 cm layer of fat-free cotton. The thimbles containing the sample were placed into Soxhlet (Shanghai Qianjian Instrument Co., Ltd) extraction chamber. The cooling water was switched on and 50 mL of diethyl ether was added to the extraction flask through extraction cylinder. The extraction was conducted for about 3 hr. The extraction flasks with their content were removed from the extraction chamber and were placed in the drying oven at 90 ^{o}C for about 30 min, cooled to room temperature in the desiccator for about 30 min and re-weighed.

The percent crude fat was calculated.

$$\%\text{Fat}=\frac{W2-W1}{W}*100\text{Where:}$$

W = weight of the sample (g), W1 = weight of extraction cups (g), W2 = weight of the extraction cup and the dried crude fat (g)

3.5.2.5. Crude fiber determination

Crude fiber content of the fermented *Teff* dough samples was determined according to approved AOAC (2000) method. A freshly weighed homogenized 2 g sample was placed into a 600 mL beaker and about 200 mL of 1.25% H2SO4 was added and the mixture was boiled gently for 30 Min. placing a watch glass over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After 30 Min of boiling, 20 mL of 28% KOH was added and boiled gently for a further 30 minute with occasional stirring. The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with a 2 mL of the distilled water.The solution was poured from the beaker into sintered glass crucible and then the vacuum pump was turned on. The wall of the beaker was rinsed with hot distilled water four times and washings were transferred to a crucible and filtered. 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. It is washed again with 1% NaOH and filtered. Finally, the residue was washed with water free acetone. The crucible with its content was dried in an electric drying oven at 130 ^oC for 2 hrs and cooled for 30 min in the desiccator and then weighed. The crucible was transferred to a Muffle Furnace (Gallenkamp, size 3) and incinerated for 30 min at 550 ^oC. Finally, it was cooled in desiccators and re-weighed.

% Crude fiber $=\frac{W2-W3}{W1} * 100$ Where: W1 = crucible weight after drying,

W2 = cruciblweight after ashing

W3 = sample weight,

3.5.2.6. Determination of carbohydrate

The carbohydrate content was determined by subtracting the crude protein, moisture, total ash, and fat from the sample's total dry weight as follows:

Total carbohydrate % =100- crude fat + moisture + crude protein + ash)

3.5.2.7. Determination of gross energy value

Gross energy was calculated according to Osborne & Voogt, 1978. The percent calories in the samples were calculated by multiplying the percentage of crude protein and carbohydrate by 4 kcal/g and crude fat by 9 kcal/g. The values were then converted to calories per 100g of the sample.

Gross energy (%) = (9 * crude fat %) + (4 * crude protein %) + (4 * crude carbohydrate %) Kcal/100g

3.5.3. Determination of mineral concentrations of fermented *Teff* dough

The minerals were determined according to the standard method of AOAC (2000). Calcium, iron, and zinc were determined by using atomic absorption spectrophotometer (AAS), About 2 g of samples were weighed into the dish and then placed on a hot plate under a fume-hood in slowly increasing temperature until smoking ceases. When the samples become thoroughly charred, the dishes were placed in a Muffle Furnace, as near to the center as possible and ashed at 550 ^{o}C for 3 hr. The dishes were removed from a muffle furnace, cooled, seen to be clean, and white in appearance. Few drops of de-ionized water and concentrated nitric acid were added, dried, and return to a Muffle Furnace when the ash appears black and not ignited well. Then dishes were checked until traces of carbon are fully ashed and then taken out of the muffle furnace placing immediately in desiccators till cooled to room temperature. The ash of the sample was made wet completely with 5 mL of 6 M HCl and was carefully dried on a low-temperature hotplate. Seven mL of 3 M HCl were added and the dish was heated on a hot plate until the solution just boils. Then it was cooled and filtered through a Whatman no.1 filter paper into a 50 mL volumetric flask retaining as much of the solids as possible in the dish.

Again 7 mL of 3 M HCl was added to the dishes and heated until the solution just boils. Then, the solution was cooled and filtered into a volumetric flask. The dishes were then washed with water, and filtered into the volumetric flask. The filter paper was washed thoroughly and collected in the flask. Since calcium is to be determined 2.5 mL of 10 % Lanthanum chloride solution were added to the flask.

Finally, the solution was diluted to the mark (50 mL) with freshly de-ionized water. The reagent blanks were prepared by taking the same amount of reagents through all steps and they were analyzed for their metal content of the sample.

Metal content (mg/100g) $=\frac{A-B*V}{10W}$

Where; - A: concentration (□g/mL) of sample solution

B: concentration (□g/mL) of blank V: Volume of extract (mL)

W: Weight of sample (g)

3.5.4. Phytochemical composition of fermenting *Teff* dough

3.5.4.1. Phytate content determination

Phytate was determined by the method described by Vantraub & Lapteva (1988). About 0.100 g of samples was extracted with 10 mL of 2.4% HCl in a mechanical shaker (Eberbach) for 1 hr. at a room temperature. The extract was centrifuged at 3000 rpm for 30 minutes. The clear supernatant was used for phytate estimation. Two mL of Wade reagent (containing 0.03% solution of $FeCl_3.6H_2O$ and 0.3% of sulfosalicylic acid in water) was added to 3 mL of the sample solution (supernatant) and the mixture was mixed on a vortex for 5 seconds. The absorbance of the sample solutions was measured at 500 nm using UV- VIS spectrophotometer (Beckman DU-64- spectrophotometer, USA).

A series of standard solution were prepared to contain 0, 4.5, 9, 18, 27 and 36 µg/mL of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3 mL of the standard was added into 15 mL of centrifuge tubes with 3 mL of water which were used as a blank. One mL of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 2 Min. The mixture was centrifuged for 30 Min. at 3000 rpm and the absorbance of the solution (both the sample and standard) was measured at 500 nm by using de ionized water as a blank.

A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation. The phytate concentration inside the samples was calculated using the following formulaand the result was expressed as phytate concentration in mg/100g.

$$phytic\ acid(\mu g/100g)\ \frac{[(AS-AB)-intercept\ ]*10}{Slope*W*3}$$

Where: - As - absorbance of the sample,

A_b -absorbance of the blank,

W-sample weight

3.5.4.2. Tannin content determination

Tannin content was determined according to the method described by (Dykes, 2019) About 2 g of the sample was weighed and mixed with 8 mL of 1% HCl solution 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 Min. 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). D-catechin was used as a standard for condensed tannin determination. A 40mg of D- catechin was weighed and dissolved in 1000 mL of 1% HCl solution in methanol, which was used as stock solution. 0, 12, 24, 36, 48 and 60 mL of stock solution was taken in a test tube and the volume of each test tube was adjusted to five mL with 1% HCl in methanol. 5mL of vanillin-HCl reagent was added into each test tube. After 20 Min, the absorbance of sample solutions and the standard solution were measured after 20 min at 500nm. The spectrophotometer was adjusted with a 1% HCl methanol blank. The tannin concentration was calculated using the following formula and the result was expressed as tannin mg/100g fresh weight.

Tannin (µg/100g) $\frac{[(AS-AB)-intercept]*10}{D*S*W}$

Where: Ab - absorbance of the blank, As - absorbance of the sample, W - sample weight, d is the density of the solution (0.791 g/mL).

3.5.4.3. Determination of oxalate content

Oxalate was analyzed using the method originally used by AOAC 2005**.**About 1 g of samples was suspended in 380 mL de-ionized water contained in a 500 mL volumetric flask; 10 mL of

6 M HCl was added and the suspension was digested at the boiling point of water for 1 hr. that followed by cooling. The solution was then centrifuged at a speed of 2500 rpm for 5 min and the supernatant was decanted and the precipitate was completely dissolved in 75 mL of 3M H2SO4 solution and shaked for 1 hr. using shaker Then filtered by using Whatman No. 1 filter paper. The filtrate was collected by using another conical flask. Aliquots of 25 mL of the filtrate and poured into 250mL conical flask and heated against 0.1 M standard KMnO4 solution to a faint pink color which persists for 1 hr. in water bath at 90 °C until pink color appeared for at least 30 sec. The oxalate content given by the relationship that 1mL of 0.1M$KMnO_4$ solution = 0.006303g of oxalate. The oxalate content was calculated by using thefollowing formula:

$$\% \text{ Oxalate} = \frac{Volume\ of\ KMno4\ consumed}{Weight\ of\ fresh\ sample} * 0.006303 * 100$$

3.5.4.4. Determination of molar ratio of ant nutrients to minerals

The molar ratio of the antinutrients (phytate and oxalate) to minerals (Ca, Zn and Fe) was predicted by dividing the mole of anti-nutrient (phytate: 660 g/mol; oxalate: 88 g/mol) to the mole of minerals (Ca: 40 g/mol; Zn: 65 g/ mol; Fe: 56 g/mol) (Norhaizan & Norfaizadatul, 2009).The calculated values of the molar ratios were also compared with the reported critical toxicity values. The ratio values determines bioavailability of the minerals considering their critical ratio limits (Phytate: Calcium <0.17; Phytate: Iron <1 and Phytate: Zinc <5) as indicated in FAO/WHO(2004)

$$Molar\ ration = \frac{[\text{mg of antinutrent} / \text{MW of antinutrient}]}{[\text{mg of mineral} / MW\ of mineral}$$

Where, MW = molecular weight

3.5.5. Determination of Antioxidant activity

Extract Preparation; - Extraction methods was followed by minor changes was used for the extraction process in which, 5 g of each of the samples were weighed out, and 50 mL of 80 % methanol (v/v) was added to each of these 5 g samples.

The mixture was put in a conical flask and covered with aluminum foil before being shaken on an orbital shaker for 4 hours at 150 rpm. (Chew *et al*., 2011).The supernatants were filtered using filter paper (Whatman-125mm x 100 circles) and used for the analysisof antioxidant activity.

The methanol was removed by using a rotary evaporator through evaporation at 60 °C and the yield was weighed, then an appropriate amount of methanol was added for each sample after multiplying and dividing the yield by 1000 and 50 respectively. The resulting extracts were stored for further analysis.

3.5.5.1. Total phenolic content determination

Total phenolic content (TPC) was determined based on the procedures described by Ferreira *et al*.,(2007) using gallic acid as a standard for the calibration curve. One milliliter of the sample was mixed with one mL of Folin Ciocalteu's phenol reagent. After 3 Min, one mL of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min after which the absorbance was measured spectrophotometrically at 725 nm using a UV-VIS Spectrophotometer (Agilent Cary Corporation, 1001, Kyoto, Japan). Gallic acid was used to construct the calibration curve. The concentration of the standard solution ranged from 0.5-100.The concentration of the standard solution ranged from 0.5–100 μg/mL.

The total phenolic content was calculated by the following formula.

$$\text{Total phenolic content (mg GAE/g)} = \frac{C*V}{m}$$

Where, C: concentration of Galic acid obtained from the calibration curve in mg/g

V: volume of sample extract in liter, m: weight of sample in gram

3.5.5.2. DPPH Assay

Determination of antioxidant activity using the 1,1- Diphenyl-2-picrylhydrazy test was carried (Etim *et al*., 2015) with minor modifications, in which 0.004 % w/v methanol - DPPH solution was prepared by diluting 0.004 g of 1, 1 – Diphenyl - 2 - picrylhydrazyl in 100 mL of methanol. The absorbance of the methanol solution of DPPH was obtained at 517 nm and recorded as blanks. Stock solution 3 mg/10 mL was used for the standard ascorbic acid and 150 µL of the crude extract were mixed with 2 mL of DPPH in methanol solution.

To prepare solutions with concentrations of 0.01, 0.02, 0.04, 0.08, 0.16, and 0.2 µg/mL, respectively, the stock solution was serially diluted.Each of the diluted solutions were incubated with 2 mL of 0.004 % methanolic DPPH at room temperature in a dark cupboard after vortexed. Using a UV-vis spectrophotometer, the absorbance for each concentration was measured at 517 nm against a blank after 30 minutes of incubation. The results were expressed as milligrams of ascorbic acid equivalent/100 g (mg AAE/100 g of flour on dry basis) by means of calibration curve.

The percentage inhibition of free radical DPPH was calculated as follows below: -

% of inhibition of DPPH radical= $((A_{control} - A\ s_{ample})/A_{blank}) * 100$

Where: $A_{control}$ =Absorbance of the control (DPPH without the test fraction)

A_{sample} = Absorbance of the sample

3.5.5.3. Determination of Ferric ion reducing antioxidant power (FRAP)

According to a procedure outlined by Benzie & Strain, 1996, the FRAP assay was carried out.. The FRAP reagent contained 20 mM(milimole) $FeCl_3 6H_2O$, 10 mM TPTZ in 40 mM HCl, and 300 mM acetate buffer (pH 3.6). The acetate buffer, TPTZ solution, and $FeCl_3 6H_2O$ solution were combined in a ratio of 10:1:1 (v/v/v) to produce the FRAP reagent .An aliquot (150 µL) of the sample in methanol was mixed with three mL of the fresh FRAP reagent and mixed properly, and then it was incubated at 37 °C for 30 min inside an electro thermal incubator (Indian model). At 593 nm, the absorbance was determined against a blank.

The results were represented as mmol Fe^{2+}/100 g extract using a calibration curve that was plotted using ferrous sulfate (5 mM) as the standard. Each sample was analyzed three times.

$$\text{mmol } Fe^{2+}/100\text{gextract} = \frac{concentration * amount\ of\ methanol\ used\ after\ evaporation}{weight\ of sample\ after\ evaporation} * 10$$

3.6. Method of analysis of baked *Injera*

Similar analytical methods had been used for analysis of baked *Injera samples* (Proximate analysis, Mineral analysis, Anti-nutritional factors, and Antioxidant activity)

3.7. Chemicals, standard solution, reagents, and equipment

The main chemicals, solution, and reagents used in the study was, methanol, H_2SO4, HCl, NaOH, $CUSO_4$, KOH, K_2SO_4, DPPH reagent, ascorbic acid, phytic acid, FRAP reagent, wade reagent, acetone, boric acid, petroleum ether, ascorbic acid, Sulfosalicylic acid, sodium nitrite ($NaNO_2$), acetate buffer, TPTZ, and $FeCl_3 \cdot 6H_2O$ and D - catechin. Chemicals of analytical grade were utilized. The main equipment used was: centrifuge, digital balance, test tubes, measuring cylinder, UV - vis spectroscopy, muffle furnace, oven, grinder, rotary evaporator, protein analyzer (Kjeldahl), fat extractor, hot plate, pH meter, crucibles, atomic absorption spectroscopy.

3.8. Determination of shelf life of *Injera*

3.8.1. Sample processing

Each fermented *Teff* dough samples (10 g pieces from all quarters of the sample) was homogenized with 90.0 mL of sterile 0.1% peptone water to prepare stock solution (Fig.6a). Stocks were serially diluted (1:10) to 10^{-5} by adding 0.1 mL of stock solution to 9 mL diluent (0.1% peptone water) in dilution tubes. Then, Potato dextrose agar (PDA) plates were prepared for yeast and mold determination. For preparation of PDA plates, about 15 mL sterile PDA media was poured on a plate, let to solidify and correctly labeled for appropriate dilutions to be used. Subsequently, 0.1 mL of diluted sample was inoculated and spread on PDA media which was prepared in advance. (Fig.6b)

3.8.2. Yeast and Mold count

Diluted *Injera* samples of the three treatments (0.1 mL) were inoculated on to (PDA) medium supplemented with 60mg/l chloramphenicol (in order to suppress the growth of bacteria) using spread plating technique and plates were incubated at 25°C for 5 days (Fig.6c). Visible colonies were counted and expressed as the total yeast and mold in colony forming units per gram (cfu/g) of samples (Kiiyukia, 2003). Counts were done starting from the first day and every two days of incubation until the 8 days and the samples were checked for the formation of mycelium. Yeast and mold counts were started when mycelium was detected on *Injera* samples during the incubation period. (Fig.6d) (CFU) can be calculated as follows; -

$$N= \Sigma c/[(1*n1) +(0.1*n2) *d$$

Where N=number of colonies per mL or g of product

Σc = sum of all plates counted, n1=number of plates in the first dilution counted

n2= number of plates in the second dilution counted

d=dilution from which the first count was obtained

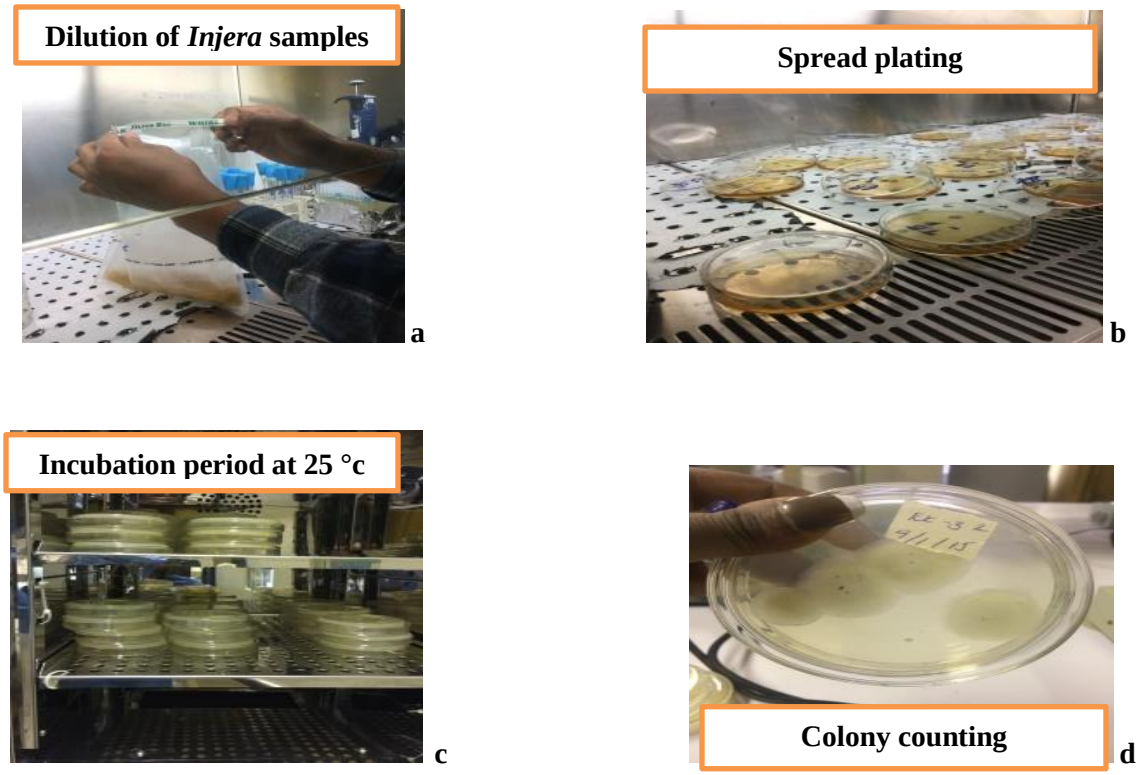


Figure 6**:** Shelf life of baked *Injera* using the three treatments

3.9. Determination Sensory Evaluation

Effect of the formulated starter culture was assessed using consumer-oriented sensory panel. A total of Six (6) *Injera* samples taken from the three treatments; T1K, T1B baked after 72 hr. fermentation, T2B, T2K baked after 48 hr. fermentation, T3K, T3B baked after 24hr. fermentation finishing the predetermined maximum fermentation stage and become ready to baking process was evaluated by a panel of consumer-oriented assessors. The samples for sensory analysis were prepared and packed as the same way done previously for other analysis, except baked on different days. The evaluation was conducted by 36 panelists (Heymann *et al*., 2012); from students and staffs of AAU, since they regularly consume it as their staple food (Fig.7).

The baked *Injera* samples were packed by using zipcoat and kept at a standard temperature until evaluation. Sensory evaluation for baked *Injera* sample was passed ethical procedure from the university to perform the analysis.(Heymann *et al*., 2012)Hence, after they acquired an orientation, equally sized portions from each sample were served and evaluated for color, texture, appearance, taste and overall acceptability using a 7-point hedonic scale score sheet. (Yetneberk *et al*., 2004). Samples were presented on identical serving trays and coded with three digit random numbers. The order of sample presentation was randomized. Clean water held at room temperature was served for cleansing mouth palate of assessors before and between testing of *Injera* samples. Samples of *Injera* being evaluated were expectorated into prepared beaker.

Figure 7: Sensory evaluation by panelists

3.10. Data Analysis

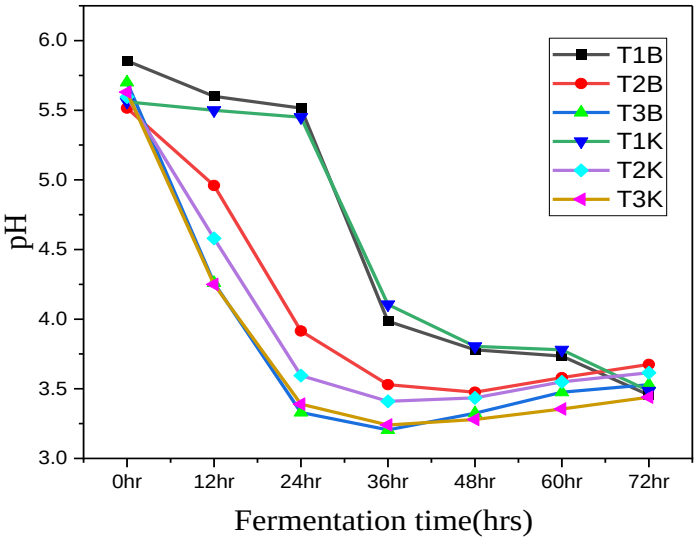
Complete randomized design (CRD) was used and data were statistically analyzed using analysis of variance (ANOVA) in order to assess the significant differences of dependent variables among samples. A least significant difference (LSD) were used to test the effects of treatments when the F-test was statistically significant at ($P < 0.05$) and Duncan's test was applied to rank the mean values of different treatments as computed by SPSS (version 23.00) software.

4. RESULT AND DISCUSSION

4.1. Physicochemical analysis of fermented *Teff* dough samples

4.1.1. pH value

According to this study the kinetics of pH value showed the same decreasing pattern in all the three treatments (Fig.8). The pH value of T1B and T1K show the sign of dropping from 0 hr. to 72 hr. whereas, the pH values of T2B, T3B, T2K and T3K was shown dropping from 0 hr. to 36 hr. and started rising at 48 hr. and 72 hr. (Fig.8). However, towards the end of the fermentation, the difference in the rate of pH change was narrowed.



Note;-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a X- axis show fermentation hr. in pH analysis is a significant difference ($p < 0.05$). **T1K;** - Kuncho *Teff* fermented by traditional *ersho*, **T1B;**- Bosete *Teff* fermented by traditional *ersho*,**T2K;**- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B;**- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K,** kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

Figure 8**:** pH analysis of fermenting *Teff* doughs from different treatments

The average mean value of pH analyzed from fermented *Teff* dough samples had ranged from (3.40-5.85) for bosete *Teff* dough and (3.44-5.59) for kuncho *Teff* dough containing treatments. The highest pH values (5.85, 5.59) fermented for 0 hr. was obtained from T1B and T1K respectively. While the lowest pH values (3.40, 3.44) fermented for 36 hr. was obtained from T3B and T3K, respectively (Fig.8).When fermentation time increased the pH value was significantly increased (Baye *et al*., 2013 and Moges,2021). In different literatures, the pH readings of *Injera* was reported with different figures for variable reasons like fermentation time, the removable supernatant liquids remedies and the amount of back slope "*ersho*" used (Yigzaw *et al*., 2004, Urga *et al*., 1997).

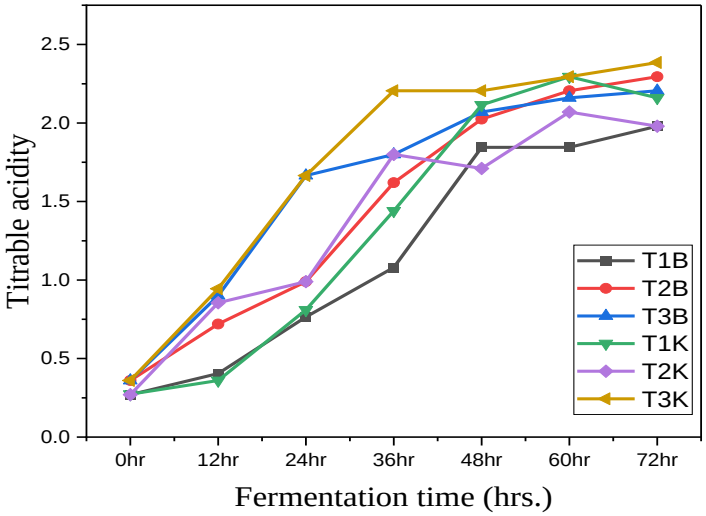
Lefebvre *et al*., (2002) stated that, when the LAB population increases, the pH value of the inoculated sourdough decreased. Similar result was reported that a pH value decrease as the fermentation time increased (Wendy, 2014). All in all, the ESA has set the pH of *Teff Injera* to be in the range 3.45 to 4.0. As a result, the values of pH obtained from the (Fig.8) were no significantly different from the set values of pH by (ESA, 2013). Cereal flours with pH of 5.0-6.2 are rich in fermentable carbohydrate was the preference fermented by lactic acid bacteria at least to a pH below 4 (Zewdu and abate,2012).Similar result was reported by Berhanu *et al*., (1982).

According to Helland *et al*., (2004) the pH decrease during fermentation is likely to be explained by the production of organic acids, mainly lactic acid. Depending on fermentation conditions such as grain variety, flour type, temperature, etc., changes in the rate of pH change is expected (Helland *et al*. ,2004). The sourness test of traditionally fermented Ethiopian *Injera* is one of the sensory attributes impacted by pH due to changes in lactic acid concentration during fermentation (Yigzaw *et al*., 2004).

In the present study, there were significant difference ($p<0.05$) among the three treatments in their pH value shown in the (Fig.8). In which (T3K, T3B) somehow show better reduction in pH value, along with (T2K, T2B), compare to (T1B, T1K). The pH decrease during fermentation is likely to be explained by the production of organic acids, mainly lactic acid.

4.1.2. Titratable Acidity

The titratable acidity content of the fermented *Teff* doughs with all treatments showed a pattern of increasing from 0 to 72 hr. (Fig 9). The average mean value of titratable acidity analyzed from fermented *Teff* dough samples had ranged from (0.27-2.29%) for bosete *Teff* doughs and (0.27-2.38%) for kuncho *Teff* doughs containing treatments (Fig.9).



Note;-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a X- axis show fermentation hr. in pH analysis is a significant difference ($p < 0.05$). **T1K;** - Kuncho *Teff* fermented by traditional *ersho*, **T1B;**- Bosete *Teff* fermented by traditional *ersho*,**T2K;**- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B;**- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K,** kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

Figure 9: The titratable acidity of fermented *Teff* doughs from different treatments

The highest TA value (2.29%, 2.3%) fermented for 72hr. was obtained from T2B and T3K , respectively. The increase in titratable acidity treatments T2B, T3B, T2K, and T3K was found higher than the treatment T1B and T1K. Generally, the result of this study indicated that the value of the titratable acidity of treatments of *Teff* doughs fermented with the formulated starter cultures was relatively higher than that of the doughs fermented with the traditional *ersho* (Fig.9). This result is in agreement with (Moges, 2021).

4.2. Proximate composition and energy value of fermented *Teff* doughs

4.2.1. Moisture content

Results of this study showed that there was a slightly difference ($p<0.5$) on the moisture content of fermented *Teff* dough samples with different treatments. The moisture content of the fermented *Teff* dough samples had ranged from (4.94-9.4%) for bosete *Teff* and (4.8-9.5%)for kuncho *Teff* dough containing treatments respectively. (Table 3).

Table 3: Moisture content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Moisture	0hr	6.355±0.346^{b}	6.020±0.381^{d}	7.910±0.070^{a}	5.825±0.035^{e}	5.710±0.055^{f}	6.225±0.162^{c}
	12hr	5.305±0.289^{b}	5.505±0.374^{a}	4.945±0.277^{c}	4.825±0.3040^{e}	4.875±0.544^{d}	4.880±0.240^{d}
	24hr	5.255±0.261^{d}	4.960±0.212^{e}	7.880±0.022^{a}	7.160±1.1455^{b}	4.8950±0.289^{f}	5.365±0.799^{c}
	36hr	6.920±0.579^{d}	6.490±0.806^{f}	6.755±0.502^{e}	7.460±0.311^{c}	7.475±0.176^{b}	8.125±0.021^{a}
	48hr	7.655±0.275^{f}	7.760±0.098^{e}	8.300±0.655^{d}	8.400±0.339^{c}	8.765±0.106^{a}	8.530±0.601^{b}
	60hr	8.585±0.530^{a}	8.480±0.919^{b}	8.345±0.544^{d}	8.135±0.289^{e}	8.580±0.967^{a}	8.420±0.791^{c}
	72hr	8.910±0.530^{c}	8.460±0.141^{e}	9.425±0.558^{b}	8.545±0.841^{d}	8.901±0.155^{c}	9.535±0.360^{a}

Note;-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K; - Kuncho *Teff* fermented by traditional *ersho*, **T1B;**- Bosete *Teff* fermented by traditional *ersho*,**T2K;**- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B;**- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K,** kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest moisture content (9.42%, 9.53%) fermented for 72 hr. was obtained from T3B and T3K, respectively. While the lowest moisture content (4.94%, 4.82%) fermented for 12 hr. was obtained from T3B and T3K respectively. Variation in fermentation time has nothing to do with moisture content. However, the relative increment of moisture content may be attributed due to a variation in the treatment during the drying process of the fermented samples. The moisture content of T3B T3K, has somehow higher value than T1B, T2B and has somehow higher value than the, T1K, T2K.This show that T3B,T3K has higher wettability than the rest .Similar observations were also made by (Yimer and Geremew ,2017).

4.2.2. Total Ash content

Results of this study showed that there was a slightly difference ($p<0.5$) on the total ash content of fermented *Teff* dough samples with different treatments. The ash content of the fermented *Teff* dough samples had ranged from 1.38-3.24 % for bosete *Teff* and 1.49-3.37% for kuncho *Teff* dough containing treatments respectively (Table 4).

Table 4: Total ash content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Ash	0hr	1.6850±0.0495^{b}	1.5900±0.056^{e}	1.8850±0.247^{a}	1.6600±0.296^{c}	1.5950±0.063^{d}	1.5900±0.028^{de}
	12hr	1.7050±0.1202^{c}	1.5000±0.014^{d}	1.6900±0.042^{c}	1.7250±0.176^{b}	1.4700±0.008^{e}	1.9100±0.169^{a}
	24hr	1.8400±0.1555^{d}	1.2500±0.282^{f}	2.0700±0.127^{c}	2.1600±0.212^{b}	1.6750±0.431^{e}	2.3100±0.127^{a}
	36hr	2.1500±0.0141^{e}	2.3000±0.353^{d}	3.0450±0.388^{b}	2.0600±0.339^{f}	2.4250±0.431^{c}	3.3000±0.098^{a}
	48hr	2.0200±0.2545^{f}	2.1400±0.212^{e}	3.1400±0.098^{b}	2.9900±0.070^{d}	3.2900±0.084^{a}	3.0200±0.042^{c}
	60hr	2.0500±0.0848^{e}	1.9600±0.008^{f}	2.3850±0.445^{b}	2.2250±0.007^{d}	2.2400±0.890^{a}	2.2650±0.275^{c}
	72hr	2.0300±0.2545^{c}	1.8800±0.098^{f}	2.0450±0.148^{c}	2.3800±0.1131^{e}	2.9200±0.169^{a}	2.1900±0.240^{b}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermenting by formulated *ersho* in incubator condition

The highest ash content was (3.2%,3.37 %) fermented for 36 hr. was obtained from T3B, T3K respectively. While the lowest moisture content (1.38 %,1.49%) fermented for 72 hr. was obtained from T2B and T3K respectively (Table 4). This was similar with they reported that there is gradually decrease in the ash contents with the increase of fermentation days (Ebadi and Reza ,1997).The study done by Gourdouvelis *et al*., (2010) also report a significant decrease of ash content after four days of fermentation. The decrease in total ash contents could be due to the fermenting microorganisms that might have used for metabolic activities (Ogbonnaya *et al*., 2010). Generally, based on the present study the total ash content pattern of the fermented *Teff* dough using the formulated starter cultures has somehow lower in total ash content than that of the traditional starter culture taken from the household.

4.2.3. Crude protein

According to the present study, there were significance different ($p<0.05$) among the treatments in their crude protein content shown in the (Table 5). The protein content of the fermented *Teff* dough samples had ranged from 12.06 -14.18% for bosete *Teff* and 12.08.-14.73 % for kuncho *Teff* dough containing treatments respectively.

Table 5: Protein content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs))	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Protein	0hr	12.735±0.374^{d}	13.1150±0.1909^{a}	12.8100±0.4101^{b}	12.1400±0.1555^{e}	12.7700±0.6788^{c}	12.1400±0.4525^{e}
	12hr	12.065±3.316^{e}	13.2700±0.1697^{b}	12.9400±0.0848^{c}	12.8050±0.0070^{d}	12.0800±0.6364^{e}	14.1600±0.3535^{a}
	24hr	12.710±1.173^{e}	13.3450±1.7465^{a}	13.1050±0.2474^{c}	12.4300±1.2727^{f}	12.8900±0.6364^{d}	13.2300±1.1596^{b}
	36hr	12.150±0.579^{e}	12.5700±0.2545^{c}	13.1450±0.5444^{b}	12.3850±0.9263^{d}	12.5750±0.5161^{c}	13.9700±2.6304^{a}
	48hr	13.225±0.063^{a}	13.1900±0.0989^{b}	12.1450±0.6010^{f}	12.5350±0.5444^{c}	12.2750±0.0919^{e}	12.4950±0.3747^{d}
	60hr	12.925±0.403^{e}	13.6200±0.7071^{c}	13.8800±0.5939^{b}	12.2850±0.2474^{f}	13.0350±1.0253^{d}	14.3500±0.1555^{a}
	72hr	13.925±1.237^{c}	13.9300±0.6081^{c}	14.1800±0.1697^{b}	12.9700±0.1131^{e}	13.4050±0.4879^{d}	14.7350± 0.8838^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,T2K;- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**, kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest protein contents (14.73% and 14.18%) fermented for 72 hr. was shown for T3B and T3K, respectively (Table 4). While the lowest protein contents (12.06% and 12.08%) fermented for 12hr. were shown for T1B and T2K, respectively. The results were in line with the range values reported by Kidist (2018). Crude protein had increased with increasing of fermentation period (Abebe *et al*., 2007). It could be as a result of the enzymatic hydrolysis of some protein inhibitors during fermentation.It may be due to death of microorganisms at the end of fermentation results, in rise of protein because yeast and LAB itself are source of microbial biomass protein. (Igbabul *et al*., 2014).

Similarly, Yimer and Geremew (2017) concluded that as fermentation period prolonged, the crude protein were found in the fermentation medium significantly increasing. Abdelhaleem *et al*. (2008) reported that the observed increment in protein content after fermentation was probably due to shift in dry matter content through depletion during fermentation by action of the fermenting microorganisms. The crude protein content pattern of the fermenting *Teff* dough using the formulated starter cultures has somehow higher protein content than that of the traditional starter culture taken from the household.

4.2.4. Crude Fat

The crude fat contents of the fermented *Teff* doughs of all treatments showed a pattern of increasing during the first 24 hr. of the fermentation time, decreasing up to 48 hr. of fermentation, and picking up again up to the end of fermentation. The samples had crude fat content ranged from (1.96 -4.93%) for bosete *Teff and* (1.89- 4.79) for kuncho *Teff* dough containing treatments respectively. (Table 6).

Table 6: Crude fat content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Fat	0hr	1.975±0.982^{d}	1.965±0.007^{e}	2.550±1.145^{a}	2.245±0.997^{c}	1.895±0.091^{f}	2.320±0.862^{b}
	12hr	3.440±0.113^{d}	2.795±0.898^{f}	3.925±0.077^{a}	3.685±0.318^{c}	3.795±0.473^{b}	3.145±0.318^{e}
	24hr	4.260±0.537^{c}	4.930±0.056^{a}	3.390±0.197^{e}	3.250±0.452^{f}	4.790±0.480^{b}	4.085±0.233^{d}
	36hr	2.695±0.077^{e}	2.715±0.091^{d}	2.865±0.021^{c}	2.485±0.063^{f}	3.105±0.007^{b}	3.221±0.169^{a}
	48hr	2.830±0.098^{e}	2.950±0.014^{c}	2.895±0.077^{d}	2.810±0.169^{f}	3.100±0.014^{b}	3.205±0.007^{a}
	60hr	3.145±0.205^{c}	3.291±0.113^{a}	3.075±0.077^{d}	3.235±0.120^{b}	2.975±0.332^{e}	3.145±0.318^{c}
	72hr	2.655±0.233^{e}	3.085±0.233^{ab}	2.830±0.000^{d}	3.070±0.042^{b}	2.905±0.148^{c}	3.095±0.120^{a}

Note:-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B;**- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**, kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest fat content (4.93%, 4.79%) fermented for 24 hr. was obtained from T2B and T3K, respectively. While the lowest fat content (1.96%, 1.89%) fermented for 0 hr. was obtained from T2B and T2K respectively.

This result agreed with values recorded by Solomon (2015). Khetarpaul and Chauhan (1989) also indicated that natural fermentation increased whereas pure culture fermentation decreased the fat content. As the fermentation preceding the fat contents of the fermented *Teff* doughs were found decreasing. In the present study, there were slightly significance different ($p<0.05$) among the treatments in there crude fat content showed in the (Table 6).In which the crude fat content pattern of the fermented *Teff* dough using the formulated starter cultures has somehow decreases than that of the traditional starter culture taken from the household. This decrease in fat contents might be attributed to the increased activities of the lipolytic enzymes during fermentation which hydrolyses fat components into fatty acid and glycerol. (Chinma *et al*., 2009).

4.2.5. Crude Fiber

The crude fiber content of the fermented *Teff* doughs all treatments showed a pattern of increasing during the first 24 hr. of the fermentation time, decrease up to the end of the fermentation time. The samples had crude fiber content ranged from (3.23-3.89%) for bosete *Teff* and (2.99- 3.79) for kuncho *Teff* dough containing treatments respectively. (Table 7).

Table 7: Crude fiber content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Fiber	0hr	3.8250±0.2192^{b}	3.1100±0.0282^{e}	3.8750±0.6151^{a}	3.0350±0.2192^{f}	3.2600±0.1838^{c}	3.2100±0.3535^{d}
	12hr	3.7850±0.2474^{b}	3.1350±0.1343^{f}	3.8050±0.2050^{a}	3.5300±0.3535^{d}	3.2050±0.0636^{e}	3.5300±0.2687^{c}
	24hr	3.7500±0.1555^{d}	3.6950±0.0919^{f}	3.7600±0.0707^{c}	3.7000±0.0565^{e}	3.8300±0.0424^{a}	3.7750±0.1202^{b}
	36hr	3.7400±0.3252^{b}	3.6450±0.0777^{c}	3.7500±0.0707^{a}	3.5000±0.6788^{e}	3.3600±0.4384^{f}	3.5000±0.0141^{d}
	48hr	3.7320±0.1414^{a}	3.5900±0.0000^{c}	3.6850±0.1484^{b}	3.4450±0.1060^{d}	3.3601±0.4832^{f}	3.4300±0.0848^{e}
	60hr	3.2300±0.2121^{f}	3.5650±0.0212^{a}	3.4300±0.0424^{c}	3.3750±0.2616^{d}	3.2850±0.5020^{e}	3.4100±0.2545^{b}
	72hr	3.2000±0.9475^{e}	3.3550±0.2192^{d}	3.3650±0.0495^{c}	3.3600±0.1697^{cd}	3.4000±0.0140^{b}	3.4900±0.3394^{a}

Note:-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition , **T3K:-** kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B;**- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest fiber content (3.79%, 3.83%) fermented for 24 hr. was obtained from T2B and T2K respectively. While the lowest crude fiber content (3.23 %, 2.99%) fermented for 72 hr. was obtained from T1B and T3K respectively.

This result show similar result with pervious study (Bultosa, 2017) The expected decrease in fiber content during fermentation could be attributed to the partial solubilisation of cellulose and hemicellulosic type of material by microbial enzymes. A previous study has reported a significant decrease of fiber contents after four days of maize fermentation (Ejigui *et al*., 2005).As indicated on the above (Table 7) there is slightly significance difference ($p<0.5$) among the three treatments. In which the crude fiber content pattern of the fermented *Teff* dough using the formulated starter cultures has somehow decreases than that of the traditional starter culture taken from the household. The result was also in line with the findings reported by Geremew (2007) ,Gebrekidan (2016).

4.2.6. Total Carbohydrate

The carbohydrate content of the fermented *Teff* doughs of all treatments showed a pattern of decreasing up to the end of fermentation. The samples had carbohydrate content ranged from (72.07 -77.31%) for bosete *Teff* and (71.06- 78.23%) for kuncho *Teff* dough containing treatments respectively. (Table 8).

Table 8: Carbohydrate content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Carbohydrate	0hr	77.250±0.905^{e}	77.310±0.254^{c}	74.845±0.912^{f}	78.230±0.509^{a}	78.030±0.763^{b}	77.725±1.449^{d}
	12hr	74.945±3.599^{e}	76.930±0.707^{c}	74.500±0.070^{f}	77.380±0.452^{a}	77.260±0.565^{b}	74.995±0.205^{d}
	24hr	73.535±1.294^{b}	73.515±1.732^{c}	73.555±0.205^{c}	75.000±0.367^{b}	75.750±0.565^{a}	75.055±0.148^{b}
	36hr	74.085±0.063^{d}	73.925±0.813^{e}	72.690±0.636^{f}	75.610±1.640^{a}	74.620±0.410^{c}	74.6850±2.99^{b}
	48hr	74.270±0.494^{a}	73.960±0.197^{c}	72.740±0.028^{f}	74.165±0.035^{b}	73.170±0.056^{e}	73.895±0.346^{d}
	60hr	73.295±0.162^{c}	72.855±0.021^{d}	72.315±0.5727^{e}	73.120±0.664^{b}	72.165±2.550^{f}	73.465±0.586^{a}
	72hr	73.045±0.685^{b}	72.740±0.254^{d}	72.070±0.8768^{f}	73.035±0.572^{c}	72.157±0.494^{e}	73.066±0.742^{a}

Note :-Results are presented as means ± SD of three replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K:-** kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B;-** Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest carbohydrate content (77.31%, 78.23%) fermented for 0 hr. was obtained from T2B and T1K respectively. While the lowest carbohydrate content (72.07 %, 71.06%) fermented for 72 hr. was obtained from T3B and T3K respectively (Table 8). When the natural fermentation time prolong the utilizable carbohydrate, values decreased this might be due to the use of CHO as an energy source by the fermenting organisms (Offiah *et al*., 2017).

Significance difference (<0.05) was existed among the three treatments as shown in the result. In which the carbohydrate content pattern of the fermented *Teff* dough using the formulated starter cultures has somehow decreases than that of the traditional starter culture taken from the household. This decrease in total carbohydrate content might be due to, particularly starch and soluble sugars are principal substances for fermenting microorganisms therefore degradation and a subsequent decrease in starch content are expected to occur. (Ejigui *et al*., 2005).

2.4.7. Energy

The energy value of the fermented *Teff* doughs of all treatments showed a pattern of increasing during the first 24 hr. of the fermentation time, decreasing up to the end of fermentation. The samples had total energy value ranged from (364.27 – 399.81%) for bosete *Teff* and (361.90-397.67%) for kuncho *Teff* dough containing treatments respectively. (Table 9).

Table 9: Energy value of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Energy (Kcal/100g)	0hr	377.715±0.7264^{e}	379.385±0.7182^{d}	373.570±0.020^{f}	381.285±0.3144^{a}	380.255±0.487^{c}	380.340±0.775^{b}
	12hr	389.160±0.1131^{c}	385.955±0.9326^{e}	393.085±0.322^{b}	382.225±0.0818^{f}	394.395±0.546^{a}	386.895±0.793^{d}
	24hr	392.920±0.3557^{d}	399.810±0.5656^{a}	397.150±0.612^{c}	388.970±0.6933^{f}	397.670±0.480^{b}	389.905±0.132^{e}
	36hr	379.195±0.7647^{a}	371.415±0.0982^{e}	368.325±0.558^{f}	374.345±0.2839^{c}	374.925±0.760^{b}	371.600±0.084^{d}
	48hr	375.450±0.6163^{a}	370.150±0.5232^{b}	366.715±0.817^{f}	372.090±0.7919^{c}	369.680±0.014^{e}	369.905±0.948^{d}
	60hr	373.185±0.8072^{b}	373.665±0.8436^{a}	372.455±0.784^{c}	371.735±0.5869^{d}	367.5750±0.104^{f}	369.965±0.589^{e}
	72hr	367.375±0.3062^{d}	373.090±0.3959^{a}	364.270±0.828^{f}	369.250±0.1254^{e}	372.245±0.099^{b}	368.535±0.647^{c}

Note;- Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K:-** kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B;-** Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest energy value (399.81.31 Kcal/ 100g ,397.67 Kcal/ 100g) fermented for 24 hr. was obtained from T2B and T1K respectively. While the lowest total energy value (364.27 Kcal/ 100g, 361.90 Kcal/ 100g) fermenting for 72 hr. was obtained from T3B and T3K respectively. (Table 9). Similar result states that natural fermentation time significantly decreased the gross energy of *Injera* (Umer, 2021).

In the present study, there were slightly significance different ($p<0.05$) among the treatments in their total energy value shown in the (Table 9).In which the total energy value pattern of the fermented *Teff* dough using the formulated starter cultures has somehow decreases than that of the traditional starter culture taken from the household.

The reduction of energy value may be due to the decreased fat content during fermentation which hydrolysis fat components in to fatty acid and glycerol and decreased carbohydrate content because of the degradation and a subsequent decrease in starch content during fermentation by fermenting microorganisms. (Umer ,2021).

4.3. Mineral concentration of fermented *Teff* dough

4.3.1. Calcium content

The calcium content of the fermented *Teff* dough treatments examined in this study is presented in (Table 10). It was shown that the calcium content of all treatments was slightly significant different from each other. The samples calcium content had ranged from (114.96-246.48 mg/100g) for bosete *Teff* and (110.98-248.30 mg/100g) for kuncho *Teff* dough containing treatments respectively. (Table10) .

The highest iron content (246.48 mg/100g, 248.34 mg/100g) fermenting for 72 hr. was obtained from T3B and T3K respectively.While the lowest iron content (114.96 mg/100g,110.98mg/100g) fermented for 0 hr. was obtained fromT1B and T1K respectively. (Table 10).

Table 10: Calcium content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Ca (mg/100g)	0hr	225.960±0.622^{c}	228.660±0.565^{b}	228.780±0.44^{a}	221.980±0.103^{f}	223.600±0.141^{d}	223.100±0.187^{e}
	12hr	227.580±0.763^{c}	229.300±0.367^{a}	229.180±0.88^{b}	222.360±0.452^{f}	224.380±0.424^{d}	223.840±0.735^{e}
	24hr	229.920±0.169^{d}	232.960±0.509^{b}	232.260±0.48^{c}	227.840±0.282^{f}	229.820±0.537^{e}	235.420±0.254^{a}
	36hr	231.020±0.198^{f}	235.280±1.470^{b}	234.380±0.48^{d}	231.640±0.678^{e}	234.480±1.244^{c}	239.040±1.414^{a}
	48hr	235.120±0.509^{f}	244.180±0.763^{a}	240.640±0.73^{c}	235.720±0.452^{e}	240.360±0.792^{d}	243.620±0.198^{b}
	60hr	238.500±0.311^{f}	244.900±0.933^{a}	243.480±0.62^{c}2	239.560±0.509^{e}	242.780±0.084^{d}	244.625±0.008^{b}
	72hr	241.160±0.862^{f}	246.020±0.084^{b}	46.480±0.90^{a}	241.240±0.169^{e}	243.740±1.951^{d}	245.300±2.291^{c}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

When the fermentation time increases the calcium content of fermented *Teff* doughs also increase. This is probably due to enhance fermentation enhance mineral absorption (Lorri and Svanberg, 1995). Based on the above result the calcium content pattern of the fermented *Teff* dough using the formulated starter cultures has somehow higher value than that of the traditional starter culture taken from the household. Increment in the calcium contents were observed with increase in fermentation time (Umar, 2022)

4.3.2. Iron content

The iron concentration of the all fermented *Teff* doughs treatments showed a pattern of increasing during the first 24 hr. of the fermentation time up to the end of fermentation. The iron content of the fermented *Teff* dough samples had ranged from (13.71-23.47%) for bosete *Teff* and (15.44-23.74%)for kuncho *Teff* dough containing treatments respectively. (Table11). The highest iron content (23.47mg/100g, 23.34mg/100g) fermented for 72 hr. was obtained from T3B and T3K respectively. While the lowest iron content (13.71 mg/100g ,15.44mg/100g) fermented for 0 hr. was obtained from T1B and T1K respectively.

Table 11: Fe content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time(hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Fe (mg/100g)	0hr	13.710±0.226^{f}	14.130±0.650^{e}	14.410±0.084^{d}	15.440±0.131^{c}	15.820±0.820^{a}	15.640±0.537^{b}
	12hr	14.940±0.198^{c}	14.740±0.084^{d}	14.680±0.509^{e}	14.340±0.198^{f}	16.240±0.329^{a}	16.210±0.254^{b}
	24hr	17.880±0.113^{f}	19.940±0.198^{b}	20.080±1.018^{a}	18.260±1.216^{e}	19.360±0.73^{d}	19.680±0.396^{c}
	36hr	19.120±0.141^{e}	20.270±0.339^{d}	20.980±0.311^{a}	19.080±0.131^{f}	20.540±0537^{c}	20.880±0.622^{b}
	48hr	21.470±0.480^{c}	21.840±0.622^{b}	21.560±0.141^{d}	20.420±0.311^{f}	21.880±0.000^{a}	21.080±0.226^{e}
	60hr	21.680±0.792^{e}	22.560±0.961^{b}	22.680±0.113^{a}	21.140±0.254^{f}	22.460±0.08^{d}	22.490±0.842^{c}
	72hr	22.760±0.069^{f}	23.450±0.113^{d}	23.479±0.422^{c}	23.120±0.071^{e}	23.680±0.08^{b}	23.740±0.367^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, T1B;- Bosete *Teff* fermented by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**, kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

Natural fermentation time also had significant effect on the iron contents of *Injera* (Umar ,2022). The above result shows that there was significance different ($p<0.05$) among the treatments in their iron content showed in the (Table 11). In which the iron content pattern of the fermented *Teff* dough using the formulated starter cultures in has somehow lower value than that of the traditional starter culture taken from the household. This is probably due to long fermentation period enhances the removal of anti-nutritional factors which are believed to be responsible for unavailability of minerals (Zewdu *et al*.,2018).

4.3.3. Zinc content

The pattern of the zinc content of all fermented *Teff* dough treatments showed a general trend of increasing from initial fermentation time to the end of fermentation. The zinc content of the fermented *Teff* dough samples had ranged from (1.13-3.28 mg/100g) for bosete *Teff* and (1.88-3.56 mg/100g) for kuncho *Teff* dough containing treatments respectively. (Table 12). The highest zinc content (3.28 mg/100g and 3.56mg/100g) of the fermented doughs was at 72 hr. and was obtained from T3B andT3K respectively. While the lowest iron content (1.13 mg/100g, 1.88 mg/100g) fermented for 0 hr. was obtained from T1B and T1K respectively. (Table 12).

Table 12: Zn content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Zn (mg/100g)	0hr	1.130±0.056^{f}	1.140±0.141^{e}	2.320±0.226^{a}	1.885±0.106^{b}	1.220±0.141^{d}	1.700±0.084^{c}
	12hr	1.780±0.141^{d}	1.140±0.141^{f}	2.240±0.169^{b}	2.000±0.113^{c}	1.700±0.084^{e}	2.320±0.113^{a}
	24hr	1.940±0.084^{d}	1.560±0.254^{e}	2.270±0.198^{b}	2.200±0.113^{c}	1.940±0.311^{d}	2.540±0.113^{a}
	36hr	2.240±0.169^{d}	1.680±0.084^{e}	2.380±0.113^{c}	2.260±0.141^{b}	2.440±0.169^{b}	2.880±0.141^{a}
	48hr	2.680±0.141^{c}	2.720±0.084^{b}	2.640±0.056^{c}	2.300±0.028^{e}	2.560±0.198^{d}	3.020±0.311^{a}
	60hr	2.700±0.169^{e}	2.880±0.084^{c}	2.960±0.018^{b}	2.580±0.028^{f}	2.840±0.056^{d}	3.135±0.134^{a}
	72hr	2.880±0.565^{f}	2.900±0.084^{e}	3.280±0 .028^{c}	3.340±0.028^{b}	3.200±0.169^{d}	3.560±0.226^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B;-** Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K:-** kuncho *Teff* fermented by formulated *ersho* in incubator condition ,**T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The results of this study showed agreements with the previous report which stated that increasing the fermentation period there was a gradual increase in zinc content. This is due to the reduction of anti-nutritional factors present in the *Teff* flour. (Baye,2014). In the present study, there were significance different ($p<0.05$) among the treatments in there zinc content shown in the (Table 12) .In which the zinc content pattern of the fermented *Teff* dough using the formulated starter cultures (T2B,T2K,T3B,T3K) has somehow higher zinc content than that of the traditional starter culture taken from the household (T1B,T1K).

4.4. Anti-nutrient composition of fermented *Teff* doughs

4.4.1. Phytic acid content

The phytic acid content of all the fermented doughs treatments has indicated sign of decreasing up to 72hr. of fermentation in all cases. The phytic acid content of the fermenting *Teff* dough samples had ranged from (210.47- 250.98mg/100g) for bosete *Teff* and (210.80-241.23 mg/100g) for kuncho *Teff* dough containing treatments respectively. The highest phytic acid content (250.98 mg/100g, 241.23mg/100g) fermented for 0 hr. was obtained from T1B and T1K respectively. While the lowest Pythic acid content (210.47 mg/100g, 210.80mg/100g) fermented for 72 hr. was obtained from T3B and T3K respectively (Table 13).

Table 13: Phytic acid content of fermented *Teff* doughs from different treatments

Parameter	Fermentation Tim e (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Phytate (mg/100g)	0hr	250.981±2.357[a]	248.586±5.909[c]	248.741±4.5[b]	241.234±2.290[d]	238.943±1.759[e]	238.755±0.199[f]
	12hr	250.907±0.243[a]	246.582±0.995[b]	244.969±1.3[c]	240.596±0.663[d]	234.366±0.896[e]	231.995±0.564[f]
	24hr	247.511±0.116[a]	232.173±0.464[c]	234.887±0.2[b]	239.122±1.161[d]	227.892±0.962[e]	228.779±0.421[f]
	36hr	240.934±0.663[a]	230.507±0.763[c]	229.333±0.5[d]	232.779±2.308[b]	222.295±4.913[e]	220.474±0.896[f]
	48hr	225.192±2.722[b]	224.539±3.552[c]	220.239±3.5[e]	229.131±03.02[a]	220.643±2.290[d]	215.140±0.962[f]
	60hr	224.173±1.759[b]	220.971±1.361[c]	216.946±0.9[e]	229.708±0.232[a]	217.084±3.087[d]	215.422±1.626[f]
	72hr	214.928±3.452[b]	212.042±2.224[c]	210.478±5.3[e]	216.826±3.950[a]	211.403±2.323[d]	210.802±4.016[f]

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, T1B;- Bosete *Teff* fermenting by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;-Bosete *Teff* fermented by formulated *ersho* in environmental condition, **T3K**;- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

This might be attributed to enzymatic hydrolysis of phytic acid by endogenous phytase , which was produced by the microorganism, may account for most of the reduction of phytic acid during fermentation (Baye *et al*., 2014). Fermentation process has capacity to reduce phytic acid in the preparation of *Teff Injera* (Fischer *et al*., 2014) This is in full agreement with (Urga *et al*.,1997) who reported that *Injera* processed from 2-3 days fermented dough was found to contain low level of phytate. It has been suggested that the decrease of phytate during fermentation could be a result of the activity of native phytase and the fermentative microflora as reported by different researchers (Shimelis and Rakshit ,2008).

Other results also found that as the fermentation time had a significant reduction effect on the phytic acid content of *Injera* (Elzien and Abdel, 2011.) Some researches stated that the loss of phytate during fermentation could be a result of the activity of native phytase and/or the fermentative microflora by different workers (Elyas *et al*., 2001),(Abdelhaleem *et al*., 2008).In the present study, there were significance different ($p<0.05$) among the treatments in their phytate content shown in the (Table 13). In which the phytate content pattern of (T3B, T3K) has somehow lower phytate content than that of (T1B, T1K) with respect to (T2K,T2B).

4.4.2. Tannin content

The trend of the tannin contents of all fermented dough treatments were shown slightly decreasing from the starting of the fermentation up to end of fermentation time (Table 14). The tannin content of the fermented *Teff* dough samples had ranged from (0.59- 0.61mg/100g) for bosete *Teff* and (0.59 -0.60 mg/100g) for kuncho *Teff* dough containing treatments (Table 14).

Table 14: Tannin content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Tannin (mg/100g)	0hr	0.605±0.029[a]	0.604±0.003[a]	0.603±0.006[a]	0.605±0.004[a]	0.606±0.001[a]	0.604±0.001[a]
	12hr	0.602±0.012[a]	0.601±0.003[a]	0.606±0.005[a]	0.601±0.005[a]	0.600±0.006[a]	0.602±0.001[a]
	24hr	0.600±0.009[a]	0.610±0.014[a]	0.600±0.012[a]	0.601±0.001[a]	0.607±0.003[a]	0.602±0.002[a]
	36hr	0.601±0.013[a]	0.600±0.033[a]	0.600±0.019[a]	0.602±0.005[a]	0.601±0.002[a]	0.601±0.001[a]
	48hr	0.601±0.044[a]	0.600±0.098[a]	0.599±0.021[a]	0.600±0.008[a]	0.600±0.004[a]	0.600±0.002[a]
	60hr	0.600±0.035[a]	0.600±0.026[a]	0.601±0.007[a]	0.600±0.006[a]	0.603±0.004[a]	0.600±0.001[a]
	72hr	0.599±0.0013[a]	0.599±0.003[a]	0.600±0.003[a]	0.599±0.002[a]	0.600±0.007[a]	0.599±0.008[a]

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermenting by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest tannin content (0.61mg/100g, 0.60 mg/100g) was detected for 24 hr. and 0hr. fermented dough samples were obtained from treatments T2B and T1K, respectively. While the lowest tannin content (0.59mg/100g,0.59mg/100g) fermented for 72 hr. was obtained from T2B and T3K respectively. Increasing fermentation time reduced the anti-nutritional factors (WHO, 2012). According to Adane *et al* ., (2013) fermentation significantly reduce the level of tannin. Reduction in tannin contents due to fermentation might have been caused by the activity of polyphenol oxidase or tanniase of fermenting microflora on tannins (Fagbemi *et al*., 2005). In the present study, there were no significance different ($p<0.05$) among the treatments in their tannin content showed in the (Table 14).

4.4.3. Oxalate content

The oxalate content of the all fermented doughs treatments has indicated sign of decreasing up to 72hr. of fermentation in all cases. The oxalate content of the fermented *Teff* dough samples had ranged from (0.21- 0.35mg/100g) for bosete *Teff* and (0.20-0.32 mg/100g) for kuncho *Teff* dough containing treatments respectively (Table 15).

Table 15: The Oxalate content of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Oxalate (mg/100g)	0hr	0.289±0.413^{a}	0.283±0.375^{b}	0.220±0.044^{d}	0.220±0.044^{d}	0.252±0.412^{c}	0.220±0.044^{d}
	12hr	0.252±0.369^{c}	0.283±0.044^{b}	0.320±0.045^{a}	0.220±0.044^{d}	0.283±0.044^{b}	0.252±0.042^{c}
	24hr	0.278±0.089^{e}	0.283±0.044^{d}	0.352±0.381^{a}	0.315±0.089^{c}	0.315±0.089^{c}	0.325±0.089^{b}
	36hr	0.278±0.089^{b}	0.220±0.044^{e}	0.278±0.089^{b}	0.246±0.044^{d}	0.272±0.044^{c}	0.314±0.044^{a}
	48hr	0.246±0.044^{d}	0.309±0.044^{a}	0.252±0.065^{c}	0.283±0.044^{b}	0.246±0.044^{d}	0.306±0.044^{a}
	60hr	0.239±0.044^{c}	0.288±0.089^{a}	0.249±0.044^{b}	0.228±0.052^{d}	0.209±0.044^{e}	0.246±0.044^{b}
	72hr	0.228± 0.089^{b}	0.249±0.044^{a}	0.218±0.089^{c}	0.208±0.057^{d}	0.201±0.396^{e}	0.200±0.932^{e}

Note:-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermenting by formulated *ersho* in environmental condition **T3K**, kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest oxalate content (0.35mg/100g, 0.32mg/100g) fermenting for 24 hr. was obtained from T3B and T3K respectively. While the lowest oxalate content (0.21mg/100g,0.20mg/100g) fermented for 72 hr. was obtained from T3B and T3K respectively. The oxalate content of the present study is comparable to the oxalate content recorded by (Habtamu, 2011). Based on the present study results show there were slightly significant differences ($p<0.05$) between formulated starter cultures and the traditional starter culture on oxalate content.

4.5. Mineral to phytic acid ratio of fermented *Teff* doughs

4.5.1. Phytate: Ca

According to the present study, there were slightly significance different ($p<0.05$) among the treatments in their phytic acid to calcium molar ratio as showed in the (Table 16). The ratio values determine bioavailability of the minerals considering their critical ratio limits Phytate: Calcium <0.17; as indicated in FAO/WHO (2004). The phy:Ca content of the fermented *Teff* dough samples had ranged from (0.05- 0.09) for bosete *Teff* and (0.05-0.08)for kuncho *Teff* containing treatments (Table 16).

Table 16: Phytic acid to Calcium molar ratio of fermented *Teff* doughs from treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Phy:Ca	0hr	0.099±0.006^{a}	0.097±0.003^{a}	0.092±0.001^{b}	0.089±0.007^{c}	0.088±0.002^{c}	0.080±0.024^{d}
	12hr	0.097±0.018^{b}	0.086±0.005^{a}	0.081±0.002^{c}	0.086±0.003^{b}	0.075±0.003^{d}	0.081±0.001^{c}
	24hr	0.068±0.006^{c}	0.068±0.008^{c}	0.067±0.001^{c}	0.084±0.005^{a}	0.074±0.005^{b}	0.077±0.003^{b}
	36hr	0.067±0.005^{c}	0.058±0.002^{d}	0.064±0.001^{c}	0.076±0.005^{a}	0.072±0.001^{b}	0.064±0.005^{c}
	48hr	0.065±0.001^{ab}	0.057±0.001^{c}	0.057±0.001^{c}	0.063±0.001^{b}	0.067±0.006^{a}	0.056±0.002^{c}
	60hr	0.061±0.002^{a}	0.057±0.002^{b}	0.055±0.002^{c}	0.062±0.002^{a}	0.058±0.001^{b}	0.055±0.003^{c}
	72hr	0.058±0.005^{a}	0.053±0.001^{c}	0.052±0.002^{c}	0.055±0.006^{b}	0.054±0.001^{b}	0.053±0.044^{c}

Note: Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* ferment by formulated *ersho* in incubator condition

The highest phytate: Ca (0.09 and 0.08) fermented for 0 hr. was obtained from T1B and T1K respectively. While the lowest phytate:Ca (0.05-0.05) fermented for 72hr. was obtained from fermented dough of treatments T3B and T3K respectively. When the fermentation time increase the phytate:Ca value of fermented *Teff* doughs become decrease (Table 16).This study result is in agreement with report which stated that starter culture fermentations were to be more effective in bioavailability of minerals due to fermentation (Bilgiçli, *et al*.,2006).

According to the present study phytic to Ca ratio had slightly significant effect ($p<0.05$) among the treatments. In which (T2K, T2B) obtain lowest phytate:Ca. Hence, it had achieved higher Ca bioavailability compare (T1K, T1B).

4.5.2. Phytate:Fe

According to the present study, there were slightly different ($p<0.05$) among the treatments in there phytic acid to iron molar ratio as showed in the (Table 17). The ratio values determine bioavailability of the minerals considering their critical ratio limits Phytate: Fe <1; as indicated in FAO/WHO (2004). The phytate:Fe ratio content of the fermented *Teff* dough samples had ranged from (0.81- 0.95) for bosete *Teff* and (0.81-0.94) for kuncho *Teff* containing treatments respectively. (Table 17).

Table 17: Phytic acid to Fe molar ratio of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Phy:Fe	0hr	0.951±0.007^{a}	0.952±0.095^{a}	0.950±0.005^{a}	0.941±0.007^{b}	0.942±0.012^{b}	0.940±0.059^{b}
	12hr	0.948±0.004^{b}	0.957±0.053^{a}	0.943±0.002^{c}	0.937±0.005^{d}	0.935±0.060^{de}	0.932±0.065^{e}
	24hr	0.943±0.001^{a}	0.906±0.056^{d}	0.897±0.003^{e}	0.924±0.014^{b}	0.920±0.011^{c}	0.919±0.026^{c}
	36hr	0.909±0.005^{b}	0.892±0.032^{c}	0.842±0.003^{d}	0.918±0.026^{a}	0.915±0.043^{a}	0.909±0.011^{b}
	48hr	0.888±0.003^{a}	0.855±0.036^{c}	0.828±0.003^{d}	0.887±0.011^{a}	0.875±0.021^{b}	0.857±0.016^{c}
	60hr	0.842±0.001^{b}	0.834±0.002^{c}	0.819±0.069^{d}	0.864±0.015^{a}	0.843±0.034^{b}	0.837±0.002^{c}
	72hr	0.834±0.002^{a}	0.822±0.028^{b}	0.812±0.021^{c}	0.837±0.021^{a}	0.820±0.012^{b}	0.814±0.036^{c}

Note ;-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B;-** Bosete *Teff* fermented by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition**, T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition ,**T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest phytate:Fe (0.95 and 0.94) fermented for 0 hr. was obtained from T2B and T2K, respectively. While the lowest phytate: Fe (0.81 and 0.81) fermented for 72hr. was obtained from treatments T2B and T2K, respectively (Table 17). When the fermentation time increase the phytate:Ca of fermented *Teff* doughs become decrease.

The results were in close agreement with (Shumoy *et al.* 2017). In addition, Fermentation is one of the most economic and effective method for reducing the content of mineral absorption inhibitors. (Adams, 1990). According to the present study Fe bioavailability better result achieved from the fermented *Teff* dough by using formulated starter cultures in environmental condition than that of the traditional starter culture.

4.5.3. Phytate:Zn

According to the present study, there were slightly significance different ($p<0.05$) among the treatments in there phytic acid to zinc molar ratio as showed in the (Table 18). The ratio values determine bioavailability of the minerals considering their critical ratio limits Phytate: Zn <5; as indicated in FAO/WHO (2004). The Phytate:Zn of the fermenting Teff dough samples had ranged from (4.94-4.98) for bosete *Teff* and (4.93-4.97) for kuncho *Teff* containing treatments respectively.

Table 18: Table The phytic acid to Zn molar ratio of fermented *Teff* doughs from different treatments

Parameter	Fermentation-time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
Phy:Zn	0hr	4.987±0.002[a]	4.985±0.001[ab]	4.982±0.001[b]	4.978±0.005[c]	4.971±0.005[d]	4.975±0.007[c]
	12hr	4.988±0.001[a]	4.983±0.008[a]	4.967±0.003[b]	4.969±0.008[b]	4.961±0.004[c]	4.963±0.003[c]
	24hr	4.984±0.001[a]	4.974±0.006[b]	4.959±0.004[d]	4.962±0.001[c]	4.955±0.002[d]	4.955±0.009[d]
	36hr	4.982±0.002[a]	4.971±0.014[b]	4.957±0.009[c]	4.957±0.008[c]	4.949±0.006[d]	4.950±0.002[d]
	48hr	4.975±0.002[a]	4.968±0.007[b]	4.948±0.001[d]	4.954±0.005[c]	4.947±0.005[d]	4.945±0.004[d]
	60hr	4.971±0. 006[a]	4.9561±0.005[b]	4.945±0.004[d]	4.950±0.007[c]	4.943±0.076[d]	4.937±0.001[e]
	72hr	4.958±0.002[a]	4.949±0.008[b]	4.938±0.003[d]	4.945±0.007[b]	4.941±0.003[c]	4.937±0.003[d]

Note;- Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K;-** Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The highest phytate:Zn (4.98 and 4.97) fermented for 0 hr. was obtained from T1B and T1K, respectively. While the lowest phytate:Zn (4.93 and 4.95) fermented for 72hr. was obtained from treatments T3B and T3K, respectively(Table 18).

The results were in close agreement with (Shumoy *et al*., 2017). Studies have shown that both spontaneous fermentations as well as fermentation with starter culture significantly reduce the content of phytic acid (Bilgiçli *et al*., 2006). Based on the present study there were slightly significant differences between fermentation time ($p<0.05$) on phytate:Zn. This indicated that a Zn bioavailability reached from fermented *Teff* dough by using formulated starter culture in environmental condition is higher, compare to the traditional starter culture (Table 18).

4.6. Antioxidant capacity of the fermented *Teff* doughs

4.6.1. Total phenol content

According to the present study, there were significance different ($p<0.05$) among the treatments in there total phenolic content as showed in the (Table 19). The Total phenolic compound capacity of the fermented *Teff* dough samples had ranged from (106.96- 342.39 mg GAE/g) for bosete *Teff* and (132.29-348.19 mg GAE/g) for kuncho *Teff* dough containing treatments respectively..

Table 19: The total phenolic compounds of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1B	T2B	T3B	T1K	T2K	T3K
TPC (mgGAE/g)	0hr	133.004±56.817^{d}	106.965±31.437^{f}	166.961±13.97^{b}	152.471±25.574^{c}	172.099±40.417^{a}	132.296±43.178^{e}
	12hr	157.619±113.00^{d}	103.955±43.290^{f}	208.368±29.24^{a}	158.240±21.765^{c}	179.495±23.423^{b}	147.772±49.764^{e}
	24hr	168.966±92.918^{e}	161.522±23.561^{f}	237.367±21.97^{a}	223.004±67.234^{c}	181.046±33.564^{d}	223.477±99.128^{b}
	36hr	179.076±86.939^{f}	185.362±12.672^{e}	241.122±44.24^{b}	228.549±15.833^{c}	212.539±29.170^{d}	284.319±127.370^{a}
	48hr	182.712±11.656^{f}	219.719±7.3467^{e}	296.473±25.38^{b}	240.362±28.225^{c}	228.086±10.858^{d}	305.132±21.296^{a}
	60hr	197.496±9.0618^{f}	276.146±63.223^{e}	311.365±68.16^{c}	291.456±54.051^{d}	312.636±44.730^{b}	327.338±30.723^{a}
	72hr	200.718±27.786^{f}	298.650±13.135^{e}	342.391±29.12^{b}	327.114±32.615^{d}	328.571±50.740^{c}	348.191±43.813^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K**;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition, **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition, **T3B**;- Bosete *Teff* fermenting by formulated *ersho* in incubator condition

The highest total phenolic compound capacity (342.39 mg GAE/g and 348.19 mg GAE/g) of fermented *Teff* dough at 72 hr. and was obtained from treatment T3B and T3K, respectively.

While the lowest total phenolic compound capacity (106.96 mg GAE/g and132.29 mg GAE/g) fermented dough at 0 hr. was obtained from treatments T1B and T3K, respectively (Table 19). A previous study (Dvorakova *et al*., 2008) showed an increase fermentation time increase in phenolic content. In this study, the result showed significant differences ($p < 0.05$) in total phenolic compound content between treatments. In which (T3B, T3K) had higher TPCs than (T1B, T1K). Therefore, food processing steps such as fermentation contribute to a better extraction efficiency of TPCs.

4.6.2. FRAP Assay

According to the present study, there were significance different ($p<0.05$) among the treatments in there FRAP Assay as showed in the (Table 20). The FRAP assay capacity of the fermented *Teff* dough samples was found ranging from (233.03 - 294.34 mmolFe2+/100g) for bosete *Teff* and (241.86-299.91mmolFe2+/100g)for kuncho *Teff* dough containing treatments respectively.

Table 20: The FRAP assay of fermented *Teff* doughs from different treatments

Parameter	Fermentation time (hrs)	Treatments					
		T1	T2	T3	T1	T2	T3
FRAP (mmolFe2+ /100g)	0hr	233.030±0.995^{f}	249.776±3.634^{b}	240.568±2.262^{e}	253.396±5.938^{a}	241.979±1.563^{d}	244.967± 4.920^{c}
	12hr	246.425±10.13^{e}	249.248±2.253^{d}	274.324±3.512^{a}	241.867±5.051^{f}	254.439±1.945^{c}	266.720±2.451^{b}
	24hr	269.883±4.254^{e}	270.013±6.523^{d}	290.108±1.321^{b}	258.356±4.177^{f}	274.093±0.981^{c}	298.189±5.809^{a}
	36hr	272.918±6.526^{d}	275.467±7.583^{c}	290.157±3.328^{b}	269.575±6.732^{e}	267.414±1.306^{f}	298.025±16.86^{a}
	48hr	280.009±2.934^{d}	289.390±10.416^{b}	288.771±6.426^{c}	274.237±3.581^{e}	274.533±3.794^{e}	290.001±1.249^{a}
	60hr	281.910±4.402^{e}	290.772±3.271^{c}	291.318±13.027^{b}	274.815±21.09^{f}	284.449±4.317^{d}	293.215±2.580^{a}
	72hr	292.401±3.699^{d}	293.147±2.236^{c}	294.742±8.093^{b}	275.537±2.528^{e}	299.918±9.897^{a}	294.867± 4.754^{b}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).

T1K;- Kuncho *Teff* fermented by traditional *ersho*, T1B;- Bosete *Teff* fermented by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermenting by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition ,**T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

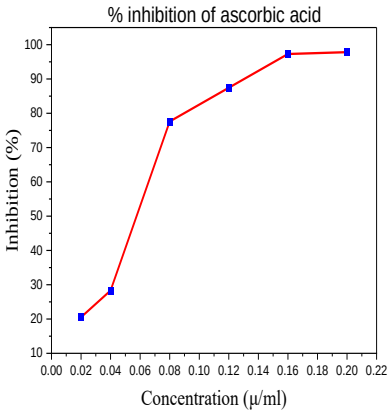
The highest FRAP capacity (294.34 mmolFe2+/100g and 299.91mmolFe2+/100g) of the fermented doughs at 72 hr. was obtained from treatments T3B and T2K, respectively.

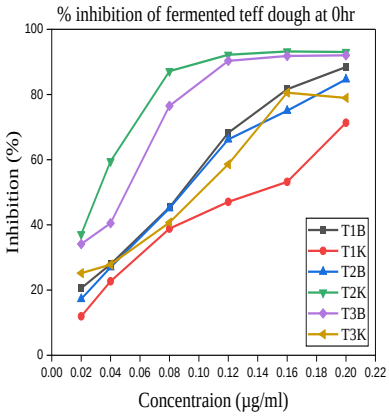
While the lowest FRAP capacity (233.03 mmolFe2+/100g and 241.97 mmolFe2+/100g) of the fermented doughs at 0 hr. was obtained from treatments T1B and T3K, respectively(Table20).These results are in agreement with (Dordevic *et al*., 2010; Habtu., *et al*.,2017).The increase in FRAP during fermentation process was also reported by (Habtu *et al*., 2017) .According to the present study Significant differences ($p < 0.05$) were observed in FRAP antioxidant capacity assays among the fermenting *Teff* dough samples. In which (T2K, T2B, T3K, T3B) showed better antioxidant capacity than (T1K, T1B).

4.6.3. DPPH assay

DPPH free radical-scavenging ability of phenolic extracts is presented in (Fig.10). During fermentation from 0–72 hr. soluble phenolic extract showed increasing % inhibition of ascorbic acid by 20% within the first 24 hr. of fermentation and slightly increase till the end of the fermentation (Fig.10).

In addition, fermentation time also affect the increase of the antioxidant capacity of the samples. The results of this study showed agreements with the previous researcher (Kotaskova *et al*., 2016; Habtu *et al*., 2017).





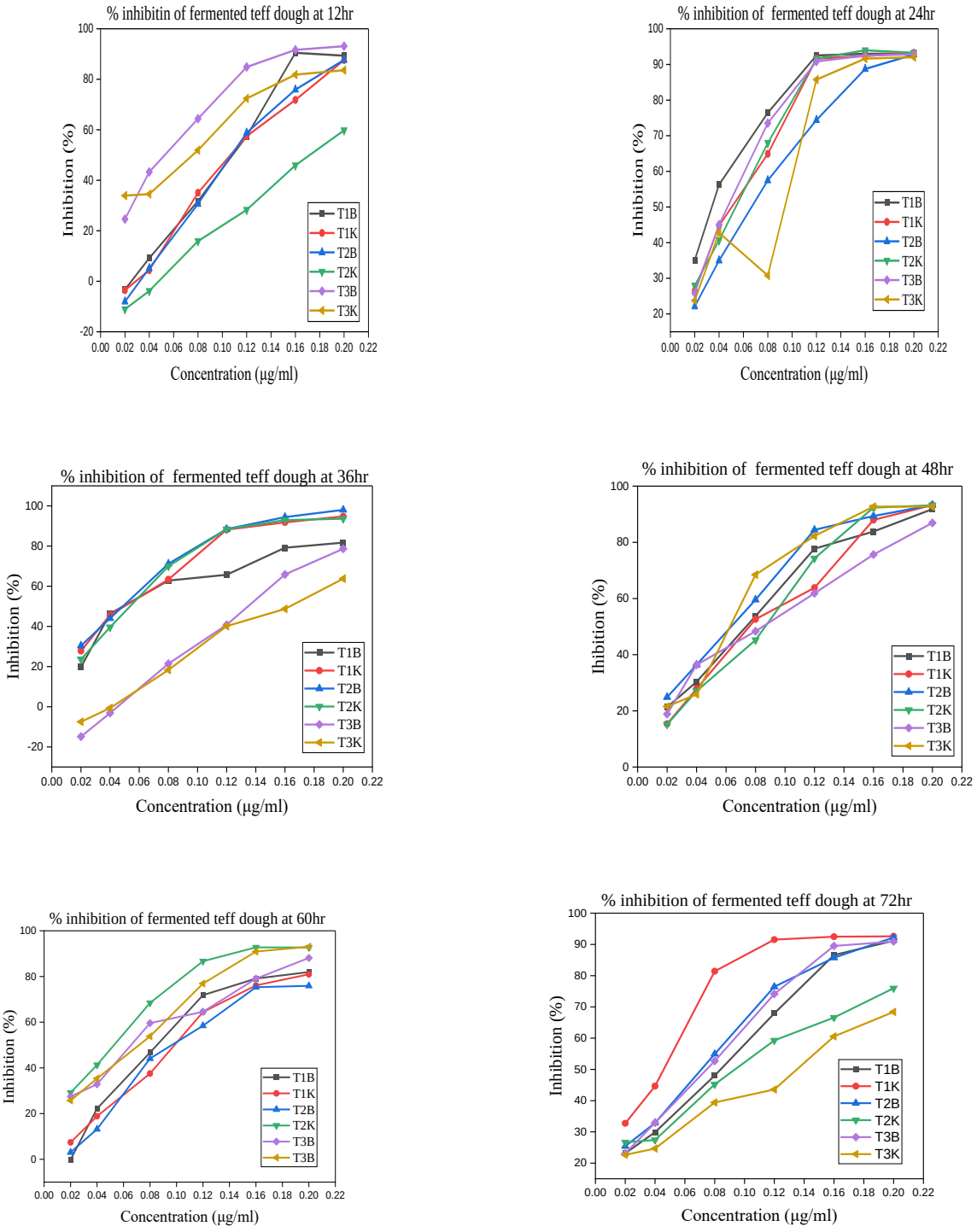


Figure 10: % inhibition of fermented *Teff* dough treatments in different time intervals

As shown in the (Fig.10) The ascorbic acid values for all treatments were between 0.02 and 0.22. Treatment (T3K, T3B) had a highest antioxidant value and showed relatively better % inhibition than other samples and (T2K, T2B) had a higher antioxidant value than traditional starter culture treatment (T1K,T1B).According to this study there was significantly different (<0.05) in DPPH radical scavenging potential among treatments during the 7 fermentation time intervals .

4.6. Proximate composition and energy value of baked *Injera*

According to the present study, there were slightly significant effect (<0.05) among the treatments on the proximate composition of baked *Injera* between the samples values (Table 21).It may due to variation in the treatment during the drying process of the fermented samples.

Table 21: Proximate composition and energy value of baked *Injera*

	Treatments					
Composition	T1B	T2B	T3B	T1K	T2K	T3K
Moisture	8.295± 0.035^{c}	8.215± 0.007^{e}	8.295±0.120^{c}	8.250± 0.014^{d}	8.390±0.028^{b}	8.470± 0.028^{a}
Total ash	2.305± 0.021^{d}	2.500± 0.042^{b}	2.370±0.070^{c}	2.255±0.021^{e}	2.500±0.042^{b}	2.940± 0.042^{a}
Crude protein	12.37± 50.163^{f}	12.575±0.163^{d}	13.535±0.247^{b}	12.490±0.651^{e}	12.755±0.728^{c}	13.900±1.344^{a}
Crude fat	3.335± 0.106^{b}	3.190±0.495^{d}	3.590± 0.070^{a}	3.265±0.148^{c}	2.485± 0.361^{f}	2.770±0.212^{e}
Crude fiber	3.405 ±0.021^{f}	3.685±0.035^{b}	3.520± 0.156^{e}	3.635±0.247^{c}	3.565± 0.389^{d}	3.940± 0.113^{a}
Carbohydrate	72.970±0.212^{e}	73.725±0.219^{c}	73.210±0.509^{d}	73.240±0.834^{d}	73.870±0.297^{b}	74.92±1.5721^{a}
Energy value Kcal/ 100g	374.275±0.757^{c}	373.51±2.930^{d}	375.290±0.410^{b}	374.305±0.601^{c}	368.87±1.520^{e}	378.210±1.004^{a}

Note ;-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).

T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermented by traditional *ersho*,**T2K;**- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermenting by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The moisture content of the baked *Injera* samples had ranged from (8.21-8.47%). The highest and lowest moisture content (8.47% and 8.21%) was obtained from T3K and T2B, respectively (Table 21). Moisture contents of all *Injera* samples were within FAO/WHO recommended safe limit (<10%) (Shevkani *et al*., 2014). The ash content indicated an estimate of the total mineral content in a given quantity of food Substance (Mishra and Chandra, 2012; Mezgebo *et al*., 2018). The total ash content of the baked *Injera* samples had ranged from (2.25-2.94%).

The highest and lowest total ash content (2.94% and 2.25%) was shown from T3K and T1K *Injera*, respectively (Table 21). The crude protein content of the baked *Injera* samples was shown ranging from (12.49-13.90%) (Table 21). The highest and lowest crude protein content (13.90% and 12.49%) was obtained from T3K and T1K, respectively. The results were in line with the range values reported by Kidist (2018), (Bekele *et al*., 1995) and (Tatham *et al*.,1996).

The crude fat content of the baked *Injera* samples was found ranged from (3.26-3.77%) The highest and the lowest crude fat contents (3.77% and 3.26%) were displayed for T3K and T1K in respectively. The crude fiber content of the baked *Injera* samples ranged from (3.40-3.94%) (Table 21). The highest crude fiber content as displayed as 3.94% for T3K and the lowest 3.40 % was obtained from T1B.The result were in closed agreement with According Geremew (2007),

The carbohydrate content of the baked *Injera* samples ranged from (72.97-73.92%) .The highest and the lowest carbohydrate contents (73.92% 72.97%) were shown for T3K and T1B accordingly. The results agreed with the values reported by (Tarekegn, 2015) and (Bultosa, 2007). The total energy content of the baked *Injera* samples found ranging from (371.30-378.87%) (Table 21). The highest and the lowest total energy contents (378.870% and 371.30%) were shown for T2K and T1K, respectively. Bultose, 2007 reported that *Teff* contains the predominantly starch (73%) the starch content of *Teff* is higher than most cereals.

The typical composition of protein, fat, ash and carbohydrate contents of *Teff* samples are given as 9.6%, 2.0%, 2.9% and 73.0%, respectively by Patricia and Lisette, (2008). These results were in close agreement with the values recorded by Solomon (2015) for moisture contents (9.69%), crude protein (12.24%), crude fat (2.69%) and total ash (2.93%) contents of Teff flour, and on the contrary with the utilizable CHO (72.37%) and gross energy (362.65 kcal/100g) values. According to this study the result there is slightly significant difference ($p<0.5$) on the proximate composition of baked *Injera.* In which (T3K, T3B) somehow showed better result than that of (T1K, T1B).

4.7. Mineral Concentration of baked *Injera*

According to the present study, there were significant effect (<0.05) among the treatments on the mineral content of baked *Injera* between the treatments samples values.

Table 22: Mineral concentration data analysis of baked *Injera* from different treatments.

Mineral content (mg/100g)	Treatments					
	T1K	T1B	T2K	T2B	T3K	T3B
Calcium	197.044±0.000^{c}	231.041±0.114^{b}	242.86±2.22^{a}	242.47±3.68^{a}	115.848± 0.103^{e}	174.288±0.271^{d}
Iron	23.139±0.025^{c}	23.428±0.044^{b}	24.789±0.013^{a}	20.415±0.029^{d}	14.493±0.0424^{f}	16.176±0.034^{e}
Zinc	1.930±0.002^{d}	1.645±0.017^{f}	2.037±0.005^{c}	2.400±0.001^{b}	1.798±0.004^{e}	2.639±0.025^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, T1B;- Bosete *Teff* fermented by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition, **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The calcium content of the baked *Injera* samples ranged from (215.04 to -242.86mg/100g) (Table 22). The highest calcium content (242.86mg/100g) was obtained from T2B and the lowest calcium content (215.04mg/100g) was obtained from T1K . According to Yu, *et al.(*2006) report *Teff* is a good source of calcium and minerals as general nutrient composition. Some other reports also showed that high calcium content may be contributed by high calcium content of *Teff* (195.2 mg/100g) (Bultosa *et al*., 2002). The iron content of the baked *Injera* samples ranged from (20.41-24.78mg/100g). The highest iron content (24.78mg/100g) was obtained from T2K and the lowest iron content (20.41mg/100g) was obtained from T1B. The total iron contents of all composite *Injera* considered in the study were shown varied from 17.73 to 25.13 mg/100 g (Baye,2014).The zinc content of the baked *Injera* samples ranged from (1.64 to 2.40mg/100g) (Table 22). The highest zinc content (2.04mg/100g) was obtained from T2B and the lowest zinc content (1.64mg/100g) was obtained from T1B.This study was found in agreement with the results indicated by (Abebe *et al*.,2007), and (Baye,2014). Based on the present study. (T3K, T3B) achieved better mineral content compare to (T1K, T1B).

4.8. Anti-nutritional composition of the baked *Injera*

According to the present study, there were significant effect (<0.05) among the treatments on the anti-nutritional composition of baked *Injera* between the treatments samples values. The phytic acid content of the baked *Injera* samples ranged from (216.36 to 238.31mg/100g). The highest calcium content (238.31mg/100g) was obtained from T1B, whereas the lowest phytic acid content (216.36mg/100g) was from T2B (Table 23).

Table 23: Anti-nutritional composition data analysis of baked *Injera* from different treatments.

Composition (mg/100g)	Treatments					
	T1B	T2B	T3B	T1K	T2K	T3K
Phytic-acid	246.82±13.870^{b}	236.36±12.61^{e}	248.85±4.62^{a}	241.31±4.290^{d}	231.02±4.35^{f}	243.96±7.50^{c}
Tannin	0.172±0.003^{b}	0.172±0.003^{b}	0.173±0.001^{b}	0.172±0.160^{b}	0.171±0.000^{b}	0.177±0.003^{a}
Oxalate	0.238±0.0446^{b}	0.189 ±0.000^{d}	0.236±0.044^{b}	0.221±0.044^{c}	0.184±0.000^{d}	0.243±0.044^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermenting by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermenting by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

Phytate degradation had been shown during baking reported in other studies (Erdman & Pneros-Schneier, 1994). (Fischer *et al*., 2014) developed starter culture to substantially degrade phytic acid during *Injera* preparation. The Tannin content of the baked *Injera* samples ranged from (0.171-0.177mg/100g). The highest tannin content (0.177mg/100g) was exhibited from T3K and the lowest tannin content (0.171mg/100g) was obtained from T2K *Injera* (Table,23). Similar report shows microorganisms somehow reduce tannin content (Woldemariam *et al*., 2019). The oxalate content of the baked *Injera* samples ranged from 0.187to 0.409mg/100g (Table 23). The highest oxalate content (0.409mg/100g) was shown for T1B and the lowest oxalate content (0.187mg/100g) was displayed for T2K. This study has similar agreement with studies which show that the use of the processing method such as baking is known to reduce some anti-nutritional factors (Bhandari and Kawabata, 2006).

4.9. Phytic acid to mineral ratio data analysis of baked *Injera*

According to the present study, there were significant effect (<0.05) among the treatments on the phytic acid to mineral ratio of baked *Injera* between the treatments samples values. The ratio value determines bioavailability of the minerals considering their critical ratio limits of phytate: Calcium <0.17 ,phytate:Fe <1 , phytate: Zn <5;as indicated in FAO/WHO (2004).

Table 24: The phytic acid to mineral ratio data analysis of baked *Injera* from different treatments

Phytate:mineral	Treatments					
	T1B	T2B	T3B	T1K	T2K	T3K
Phytate :Ca	0.056±0.004^{d}	0.053±0.005^{d}	0.074 ± 0.006^{b}	0.066± 0.005^{c}	0.054±0.006^{d}	0.106±0.001^{a}
Phytate:Fe	0.734±0.005^{c}	0.763±0.002^{a}	0.660 ± 0.003e	0.740±0.002^{b}	0.695±0.009^{d}	0.647±0.012^{f}
Phytate:Zn	4.880±0.006^{e}	4.951±0.006^{c}	4.988 ± 0.001^{a}	4.883± 0.007^{e}	4.932±0.006^{d}	4.978±0.004^{b}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermenting by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermenting by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition ,**T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

According to this study result shown in (Table 24) the phytate:Ca of the baked *Injera* samples was ranged from (0.05 to -0.10) The highest (0.10) was shown for T3K and the lowest (0.05) was displayed for T2B. The phytate:Fe of the baked *Injera* samples ranged from (0.69 - 0.94).The highest value (0.94) was obtained from T3K and the lowest value (0.69) was obtained from T2K .The phytate:Zn ratio of the baked *Injera* samples ranged from(4.93 - 4.98. The highest result (4.98) was obtained from T3B and while the lowest (4.93) was obtained from T2K. Such reduction in phytate contents may increase the amount of soluble iron, zinc and calcium and thereby increase their bioavailability (Blandino *et al*., 2003). Based on the above result there were significant effect ($p<0.5$) on the bioavailability of minerals between treatments. In which somehow the formulated starter culture higher mineral bioavailability than the traditional starter culture collected from household.

4.10. Antioxidant capacity of the baked *Injera*

According to the present study, there were significant effect (<0.05) among the treatments on the anti-oxidant potential of baked *Injera* between the treatments samples values. The Total phenolic content of the baked *Injera* samples was shown ranged from (170.7 GAE mg/100g to 394.0 mgGAE /100g) (Table 25).

Table 25: Antioxidant capacity of baked *Injera* from different treatments

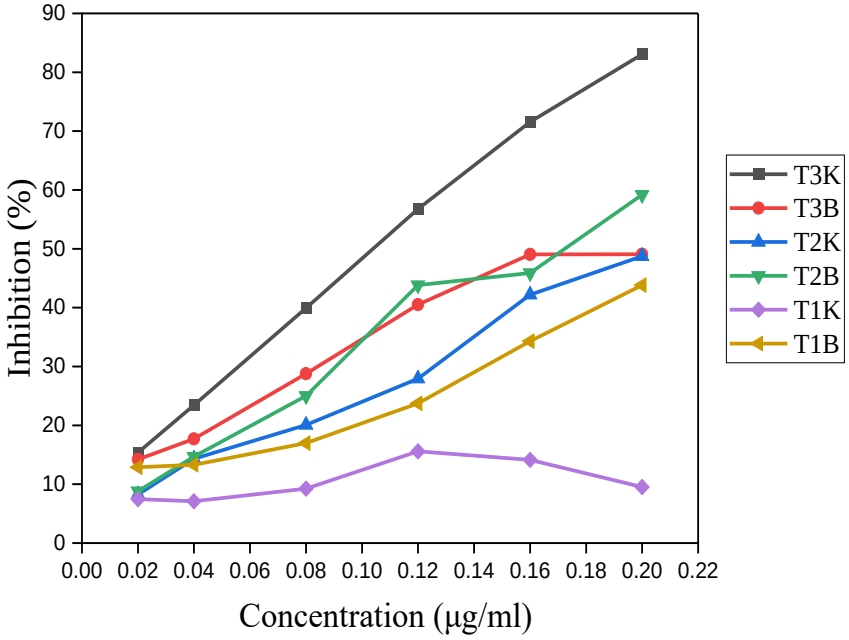
Method of assay	Treatments					
	T1B	T2B	T3B	T1K	T2K	T3K
TPC (GAE mg/100g)	177.3±21.2^{e}	360.10±67.30^{c}	394.0±203.0^{b}	170.7±29.70^{f}	243.90 ± 67.8^{d}	425.10±174.10^{a}
FRAP((mmolFe2+/g)	260.06±6.43^{e}	327.30 ± 1.04^{d}	276.56± 6.61^{b}	226.02±0.79^{f}	292.54±5.55^{c}	348.99±16.30^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermenting by traditional *ersho*, **T1B**;- Bosete *Teff* fermenting by traditional *ersho*,**T2K**;- Kuncho *Teff* fermenting formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermenting by formulated *ersho* in environmental condition, **T3K**:- kuncho *Teff* fermenting by formulated *ersho* in incubator condition
T3B;- Bosete *Teff* fermenting by formulated *ersho* in incubator condition

The Total phenolic content of the baked *Injera* samples was shown ranged from (170.7 GAE mg/100g to 394.0 mgGAE /100g) (Table 25). The highest total phenolic content (394.0 GAE/100g) was obtained from T3B and while the lowest total phenolic content (170.1 GAE mg/100g) was obtained from T1K. This study showed agreement (Shumoy and Raes, 2016).

The radical scavenging capacity of the phenolic is dependent on their structure and composition (li *et al* 2021). The FRAP assay content of the baked *Injera* samples was found ranging from (226.02 - 376.56 mmolFe2+/g) as shown in the (Table 25). The highest FRAP assay content (376.56 mmolFe2+/g) was obtained from T2B and while the lowest FRAP assay content (226.02mmolFe2+/g) was obtained from T1K. The findings also in agreement with report of Moore *et al*., 2009.The DPPH assay content of the baked *Injera* samples ranged from 0.02to 0.22% (Fig.11). The highest % inhibition of ascorbic acid (0.22%) was obtained from T3K and while the lowest % inhibition of ascorbic acid (0.02%) was obtained from T1K.

Results of the present study are consistent with the previous study which exhibited the antioxidant capacities contributed by Li *et al.*,(2021). The present study revealed that the ready to consume *Injera* baked by using the formulated starter culture shows significant effect (<0.05) on the increasing antioxidant capacity. This is may be due to the fermenters that used for the fermentation process because fermentation has significantly improved antioxidant potentials of Injera. (Oliveira *et al*., 2012).

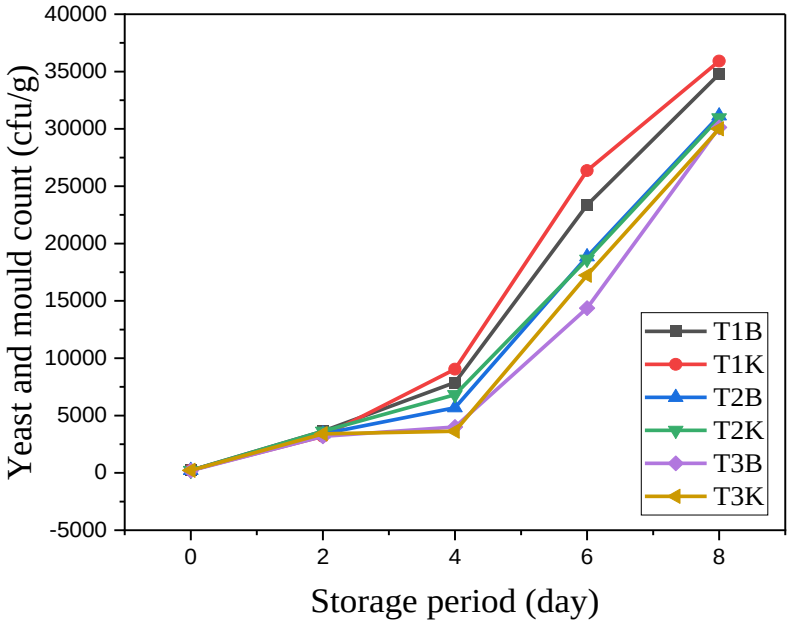


Note;-Results are presented as means ± SD of twice replications. Mean values with different superscripts in X- axis show concentration(µg/ml. in % inhibition is a significant difference ($p < 0.05$). **T1K;** - Kuncho *Teff* fermented by traditional *ersho*, **T1B;**- Bosete *Teff* fermented by traditional *ersho*,**T2K;**- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B;**- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K,** kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

Figure 11: % inhibition of baked *Injera* in different treatments

4.11. Shelf life of *Injera*

The results on the counts of yeasts and molds of baked *Injera* was prepared by using the formulated starter culture fermented under laboratory condition using incubator for tretments incubator condition (T3K, T3B), in ambient temperature condition (T2K, T2B) and by using the traditional household starter culture as a control (T1K,T1B). Then stored at a given storage condition for a total of 8 days .



Note;-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a X- axis show storage period (day) in yeast and mould count (cfu/g) analysis is a significant difference ($p < 0.05$). **T1K;** - Kuncho *Teff* fermented by traditional *ersho*, **T1B;**- Bosete *Teff* fermented by traditional *ersho*,**T2K;**- Kuncho *Teff* fermented by formulated *ersho* in environment condition, **T2B;**- Bosete *Teff* fermented by formulated *ersho* in environmental condition **T3K,** kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

Figure 12: Colony forming units of yeast and mold/g of baked *Injera* with respective treatments at different storage intervals**.**

The Fig.12 showed a pattern in which the counts of the yeasts and molds showed slight growth during the first two days and then after rapidly increased as the storage period continued up to 8 days.

The shelf life analysis result of baked *Injera* samples had ranged from ($3.1*10^3$-$4.1*10^4$cfu/g). The highest mold yeast count ($4.3*10^4$ cfu/g) was obtained from TB in day 7. While the lowest mold count result ($3.1*10^3$ cfu/g) was obtained from T3B the first storage day (0 day).

The patterns of rising yeast and mold colony counts were observed to be relatively exponential. This is due to that, molds are strictly aerobic microorganisms widely spread in nature. Thus, the limitation of oxygen on their surrounding environment suppresses their growth. For the retardation of mold growth and attainment of long shelf lives, the levels of residual O_2 must be kept below 1% (Guynot *et al*., 2003). In similar study the shelf life of *Injera* was determined by conducting the yeast and mold counts (Girma *et al*., 2013). As displayed on the (Fig.12) the numbers of yeast and mold colony counts of *Injera* at the day of baking for all the treatments were $2*10^1$ cfu/g.

As the microbial result shows, the numbers were raised form each baked *Injera* samples fermented by using formulated starter cultures as the number of storage period increased. Relatively, maximum numbers of yeast and mold colonies was observed in the sample which had been fermented by using traditional starter culture.

Fungi are the most common spoilers in bakery products. Commonly a shelf life of 3-4 days may be expected when they are unpreserved (Ryan *et al*., 2008). As shown in the (Fig.12) at ambient storage temperature the maximum yeast and mold count on 7day was observed in the T1B with yeast and mold count of $4.3*10^4$ cfu/g. The colony count for the samples (T1K, T1B) at the day of baking was $2*10^1$ cfu/g which increased from ($7.0*10^3$ cfu/g to $2.6*10^4$cfu/g) for T1K and from($7.8*10^3$cfu/g to $2.3*10^4$ cfu/g) for T1B at the third and sixth days of storage period respectively. Also the maximum number of yeast and mold colony following the control samples were (T2K, T2B) which reached to ($3.2*10^4$ cfu/g , $3.1*10^4$ cfu/g) respectively .

As the microbial result shows the minimum yeast and mold count on 7day was observed in the T3B, T2B with yeast and mold count of ($3.1*10^4$ cfu/g, $3.1*10^4$ cfu/g) respectively .In addition (T3B,T2B) samples finished there fermentation time in 24 hr. in which fermentation takes place in incubator using the formulated starter culture this lied's to reach minimum yeast and mound count and longer shelf life of *Injera* at the time of storage.

Among all factors that affected microbial growth, temperature is one of the most important factors directly affecting the growth of microorganisms in foods.

Evaluating the effect of temperature on microbial growth is of paramount important in predicting microbiology and shelf life of a product (Huang *et al*., 2011).In this study, the baked *Injera* prepared by using the formulated starter culture (T3B, T3K, T2B, and T2K) had shown better significant effect($p<0.05$) in the mold and yeast counts, this reduced the number of spoilage microorganism and thereby storage period is higher than *Injera* prepared with the traditional starter culture (T1K, T1B). The shelf life of *Injera* baked using the formulated starter cultures (T2K, T2B, T3K, T3B) was stays for 5 days while shelf life of *Injera* baked by using the traditional starter cultures from house hold stays for 3-4 days.(Fig.12)

According to Zewdu and Abate (2012) studies *Penicillium and Rhizopus* were more dominant in spoiling *Injera* at lower temperature (16- 200 ^{0}c), while *Aspergillus niger* grow much faster as the temperature gets higher (25-320 ^{0}c). Depending up on the type of mold that dominated the *Injera* samples at the given condition there might be a variation in the degree of invasion. None of the molds grew when the temperature was kept at 4°c. (Zewdu and Abate,2012) Whereas, the yeast and mold colony count were vastly reduced due to baking. At the day of baking, yeast and mold count of *Injera* was found to be $2*10^1$ for all treatment samples. (Zewdu and Abate,2012)

4.12. Sensory evaluation of baked *Injera*

Table 26: Sensory evaluation of baked *Injera* from different treatments by panelists

	Treatments					
Sensory attributes score	T1B	T2B	T3B	T1K	T2K	T3K
Color	6.433±0.516^{bc}	6.343±0.816^{c}	6.667±0.516^{a}	6.500±0.837^{bc}	6.633±1.169^{ab}	6.510±0.548^{bc}
Aroma	6.667±0.516^{a}	6.500±0.548^{b}	6.500±0.548^{b}	6.667±1.169^{a}	6.427±0.408^{b}	6.567±1.506^{b}
Taste	6.500±0.548^{b}	6.467±0.753^{b}	6.260±1.673c	6.667±0.516^{a}	6.500±0.548^{b}	6.333±0.516^{c}
Texture	6.500±0.548^{c}	6.333±1.862^{d}	6.900±0.000^{a}	6.657±0.516^{b}	6.433±1.602^{d}	6.667±0.753^{bc}
Overall acceptability	6.560±0.158^{ab}	6.407±0.557^{c}	6.515±0.262^{ab}	6.620±0.632^{a}	6.495±1.058^{bc}	6.425±0.379^{c}

Note ;-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$). **T1K**;- Kuncho *Teff* fermented by traditional *ersho*, T1B;- Bosete *Teff* fermented by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, T2B;- Bosete *Teff* fermented by formulated *ersho* in environmental condition T3K, kuncho *Teff* fermented by formulated *ersho* in incubator condition T3B;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

The pattern of results (color, taste, texture, aroma) from the evaluation of panelists indicated that almost all products including the control were scored above 5 out of 7 (Table 26). Sensory evaluation is a scientific discipline that analyses and measures human responses to the composition of food and drink using one or more of the five human senses - taste, smell, touch, sight, and hearing (Ghebrehiwot *et al*., 2016).In the current study, a panel of 36 panelist was described their degree of sensory acceptance to the *Injera* .Samples were baked after reached expected fermentation time in which *Injera* fermenting by using the formulated starter culture in incubator (T3K,T3B) baked after 24hr., in environmental condition (T2K,T2B) after 48hr. fermentation and traditional starter culture taken from household (T1K,T1B) after 72hr. fermentation time.

The color of *Injera* is one of the most important parameters which mostly catch the first look of the consumers.As clearly observed in the (Table 26) all the 6 *Injera* samples have relatively similar score in color observation in which baked *Injera* baked by using formulated starter culture have relatively better degree of liking in color than that of the baked *Injera* fermented by traditional starter culture.

In addition to its color, appearance is another important factor which refers to the quality of the eyes (cells) of the honeycomb-like structure of the top surface of *Injera* formed during cooking due to escaping CO_2 bubbles (Yetneberk *et al*., 2004). Aroma is also another important parameter which determined by smell in this study all of the treatments have positive result on the degree of liking along result goes T1K>T1B>T3K>T3B>T2B>T2K.Out of the 6 *Injera* samples, Sample T1K,T1B were the most preferred taste, scoring a 90% positive (like) response and the highest mean rating followed by T2K with a mean rating of 6.5 Pair-wise T2B and T3K followed by 6.49, 6.33, respectively .Least mean rating is scored by T3K with 6.33 liking score.

In this study (T1K,T1B) show relatively higher result taste wisely than that of the (T2K,T3K,T2B,T3B). The taste of *Injera* is associated with the sweet, sour and bitter sensations triggered in the mouth by contact with the *Injera* (Ghebrehiwot *et al*., 2016). In Ethiopia where *Injera* is consumed as a staple food ,Texture is also another important parameter which determined by touch and refers to the degree of roughness, smoothness, hardness or softness. According to the this study *Injera* samples; T3B,T3K,T1K,T1B,T2K,T2B shows degree of liking scores of 6.9, 6.66, 6.65, 6.5, 6.43, 6.33 respectively in terms of texture. In this experiment, results showed that all of the three treatments got positive response from the panelists and the samples scored higher degree of liking in overall acceptability.

Beside the complex nature of fermentation and development of proper starter culture the quality and the nature virtue of *Injera* may need further analysis meanwhile this study aimed that the formulated starter culture (T3K,T3B,T2K,T2B) have to become organolptically acceptable by panelist because along with the nutritional quality in order to be make standardized product and also to be used for commercial purpose in the future. Therefore, the *Injera* baked by using formulated starter culture is a preferable for the objective of retaining sensory quality as a product.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The results obtained indicated that ($p < 0.05$), there were significant difference on the physicochemical analysis between treatments. In which (T3K,T3B) show lower pH value and higher TA value compared to (T1K,T1B).This result predict that the fermented *Teff* dough using formulated starter culture can reduce the fermentation time by lowering the pH value and in which pH is the effective parameter in the fermentation process.

The proximate composition of fermenting *Teff* dough and ready-to-consume *Injera* of all treatments are nearly the same in which fermentative microbes (starter culture) show slightly significance effect between the treatments. Variation in fermentation time and starter culture by itself has no that much higher effect on the composition of matter. However, the relative increment of nutritional composition may be attributed due to a variation in the treatment during the drying process of the fermented samples. The proximate and mineral composition was increased with slight depreciation.

Fermented *Teff* dough (T3B,T3K,)and baked *Injera* (T3K,T3B) accompanied by having the higher in phenolic content , higher % of inhibition and iron scavenger compere to (T1K,T1B) .In addition from the study, it was also observed that the fermentation processing methods were lowering the phytate content and improving the bioavailability of essential minerals in *Teff* dough and *Injera* samples .

. The finding suggests that the sensory acceptability of almost all *Injera* samples scored better value in terms of appreciable taste, texture, appearance and/or overall acceptability attributes. Generally, from the results of this study, it was observed that formulated starter culture ‘*’ersho’* had a significant effect($P<0.05$) on reduction of the fermentation time, improve consistency, maintaining nutritional value and quality of *Injera* .

5.2. Recommendations

Recommendations for further work to enhance more concerned researches on the following:

- Further study on packaging the formulated starter culture *"ersho"* for commercial purposes and promoting its production is recommended.
- More intensive work is requiring in shelf life analysis on dough containing formulated starter culture in solution form, by using instrument like a lyophilizer to predict the packaging shelf life of the formulated starter culture *"ersho"*.
- It is also recommended that the reconsideration of applying this formulated starter culture on other cereal flours that used for *Injera* making process.
- Researches needed on to identify other factors which influence the quality of *Injera* rather than fermented microorganisms. Studing the fermentation dynamics using the modern statute of technology. (Metabolomics and metagenomics.Then based on the metagenomics and metabolomics data, proper solution of defined starter culture is recommended.
- Further study on toxicology and safety of starter culture using invivo and WGS is required before commercialization of *"ersho"*
- Change in the microbite ,vitamin synthesis need further study
- Acid tolerance test and AMR should be evaluated
- Additional research is also required to determine the shelf life of *Injera* in various storage environments and at various temperatures right after baking of *Injera.*

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APPENDIX

Appendix A: - Sensory evaluation form

Directions:- Check one rating for each of the following: Appearance, Flavor, Texture, Smell, and Overall Acceptability on a scale from 1 to 7, ‘’ 1’` being “dislike extremely , ‘’7’’ being “like extremely” to with resulting statistical indices on the *Injera* fermented with the formulated starter culture testing for texture,appearance ,taste and overall acceptance.

Panelist name: -				Product code: -		
Score		Sensory attributes				
		Color	Aroma	Taste	Texture	Overall acceptability
7	Like very much					
6	Like moderately					
5	Like slightly					
4	Neither like nor dislike					
3	Dislike slightly					
2	Dislike moderately					
1	Dislike very much					

Other note :----------

-

Appendix B: Manuscript

Addis Ababa University

Center for Food Science and Nutrition

Cover letter

To: Editor-In-Chief: International Journals of Food Science and Biotechnology

June, 2023

Dear Editor In-Chief,

I wish to submit an original research article entitled ***"****Effect of formulated starter culture "ersho" on the biochemical profile of fermented Teff dough, sensory quality and shelf life of Injera"* I confirm that this work is original and has not been published and not considered for publication elsewhere. A cross sectional study was carried out to evaluate the effect of the formulated starter culture in terms of nutritional quality, shelf life and sensory evaluation compare to the traditional starter culture taken from households. Fermentation is one of the most economical methods of producing and preserving foods. *Injera* preparation is typically performed at the household level which often carried out using traditional practices. Previous experiences indicated the presence of poor yield, sourness due to long hours of fermentation, insufficient nutritional value, and lack of consistencies. The prospect of applying starter cultures will become attractive as reduction of costs (e.g. energy), reduced fermentation times, reduced risk of spoilage, improved process control, improved sensory quality, improved safety attributes and reduced preparation procedures for the final product. This could be contained by using biologically active components that can have a positive impact on quality, consistency and shelf life of the ready to consumed product. I believe that the findings presented in my paper will appeal to the specific scientists who subscribe to *International Journals of Food science and Biotechnology.* I Hope the findings will provide baseline data and information on the evaluation of the formulated starter culture on three physicochemical analysis, nutritional quality, shelf life and sensory acceptability.

I have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to me at: faniamen7@gail.com

Thank you for your consideration of this manuscript.

Sincerely, Amen Leye (MSc. student)

Institute of Biotechnology, Center for food science and Nutrition, Addis Ababa University

Effect of formulated starter culture '*ersho*' on the biochemical profile of fermented *Teff dough*, sensory quality and shelf life of *Injera*

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Abstract

Injera is one of the most popular fermented foods among Ethiopians. However, occasional failures do occur in the fermentation that leads to inconsistencies in the quality of *Injera* and it is difficult to set certain standards for *starter cultures*. This study aimed to evaluate the effect of formulated starter cultures (*Aerobic mesophiles, molds, yeasts, lactic acid bacteria (LAB), non-pathogenic coliforms*) on biochemical profile of fermented *Teff* dough, sensory quality and shelf life of *Injera*. Experimentation and processing of data were performed on 2 *Teff* varieties (Kuncho and Bosete), 3 Treatments (3% traditional household starter culture(T1), 3%formulated starter culture in environmental condition (T2) and incubator condition, (T3)) and 7 Fermentation time intervals (0 hr. ,12 hr. ,24 hr. ,36 hr., 48 hr., 60 hr. ,72 hr.). Among the three treatments T3K and T3B had obtained least pH value (3.20, 3.24), Besides, T2K, T2B had obtained lower pH value (3.43, 3.47). Despite the T1K, T1B had highest pH value (3.78,3.78), respectively from fermented *Teff* dough samples. Among baked *Injera* samples T3K and T3B had obtained highest Fe bioavailability (0.64, 0.66), phenolic content (425.10 mgGAE/g, 394.01 mgGAE/g) respectively. Besides T2K, T2B had obtained Lower Fe bioavailability (0.69,0.76), and total phenolic content (243.9 mgGAE/g ,360.10 mgGAE/g) respectively. Despite the T1K, T1B obtained lower Fe bioavailability (0.74, 0.73), and total phenolic content (170.71 mg GAE/g, 177.3 mgGAE/g) respectively. All the three treatments showed yeast and mold counts that ranged from ($3.1*10^3$-$4.1*10^4$ cfu/g). Based on the results obtained it could be concluded the formulated starter culture (T2, T3) showed significant effect ($p<0.05$) on physicochemical properties, mineral bioavailability, improvement of storage period of *Injera* extend in to 5 days and provide consistency of starter cultures in the preparation of *Injera*.

Key words: - *Isolates, Starter cultures, fermentation, isolates*

1. Introduction

Fermentation is one of the most economical methods of producing and preserving foods. It provides a way to preserve food products, to improve organoleptic properties by producing different flavors of foods, improving the nutritive value of foods and to reducing toxic substances from foods (Thiele *et al*., 2002). *Injera* is the most popular fermented food among Ethiopians. It is typical fermented pancake-like bread that is thin, flat, and many-eyed. Injera is soft fermented baked bread that is typically obtained after cereal flour has been fermented for 24 to 96 hours, depending on the ambient temperature (Askal and Kebede,2013).In fermentation processes, various starter cultures and even back slopping are extensively used. A starter culture is a preparation that contains a large number of various microorganisms that can be added to speed up the fermentation process. A typical starter, is substrate-adapted that allows better control of a fermentation process and predictability of the products (Holzapfel, 1997).Some LAB and yeast strains found in fermented foods are capable of decomposing anti-nutritional substances including phytic acid and phenolic compounds (Baye *et al*.,2014).

According to Mihrete (2019), the time necessary for Injera dough fermentation can be influenced by a variety of factors such as the microbial flora of '*ersho'* and flour, fermentation temperature, and the cleanliness of the container used. The predominant organisms identified were Lactobacillus, Bacillus and Yeasts. The fermentation process was characterized by the fall in pH from 5.0 to 4.2 and rise in the titratable acidity from 0.20 to 0.50% during 96 hr. of fermentation (Zewdie *et al*., 1997). Lactic acid bacteria were responsible for the acidic character of the *Teff* dough fermentation. (Guandalini,2006). Although the major quality attribute of a good *Injera* is its slightly sour taste, which is due to the acidic nature of *Injera* (Zegeye, 1997). Its acceptance and palatability is also determined by its desired texture (stalling) and appearance. Unfortunately, the shelf life and those quality attributes of *Injera* not stayed longer only 3 to 4 days (Zewdu and Abate, 2012). In Ethiopia, fermented food preparation is predominantly a household phenomenon. Every household appears to process food starting from raw ingredients to the final products. Housewives bake *Injera* every 2 - 3 days, and know the usefulness of the starter culture. However, occasional failures do occur in the fermentation that leads to inconsistencies in quality of *Injera* (Askal and Kebede ,2013).

However, previous experiences indicated the presence of poor yield with, sourness due to long hours of fermentation, insufficient nutritional value, and lack of homogeneity. This could be contained by using biologically active components that can have a positive impact on quality, consistency and shelf life of the ready to consumed one (Ashenafi, 2002).

Adeba (2021) has recently reported that consortia of microorganisms including *Enterobacteriaceae, aerobic mesophiles, mold, yeast, lactic acid bacteria (LAB), and nonpathogenic coliform* involved in *Teff Injera* dough fermentation. Based on this finding, This study is aimed to evaluate the effect of this formulated starter culture *''ersho'* 'on the biochemical profile, mineral bioavailability of fermented *Teff* dough ,sensory acceptability, and shelf life of *Injera***.**

2. Materials and Methods

2.1. Raw material collection

The raw materials Kuncho –white *Teff* (DZ-Cr-387), and Bosete –white *Teff* (DZ-02-161) grain varieties were collected from Debrezeit Agricultural Research Center (DZARC). The newly formulated starter culture isolates were collected from National Agricultural Biotechnology Research Center (NABRC). As a part of Injera Project, these microbial consortia were isolated, characterized, formulated and maintained at NABRC (Adeba, 2021). The traditional starter culture 'Ersho'(source of yeast and other microorganisms) collected from a household. All the collected raw materials and the microbial isolates (starter cultures were stored carefully until the appropriate and respective laboratory analyses were conducted.

2.2. Study setting

The study was laboratory-based experiments. The experiments were conducted on fermented of Teff doughs and ready-to-consume Injera samples. The experiments were conducted at Food Science and Nutrition laboratory, Addis Ababa University and Animal products, veterinary drug and feed Quality Assessment center (APVDFAC).

The prepared samples were kept in safe place until analysis takes place. A total of 42 sample in duplicate (84 samples) were tested from fermented Teff dough samples for biochemical profile laboratory analysis and total of 6 samples were tasted from baked Injera for biochemical profile, shelf life study, sensory evaluation of baked *Injera* samples.

2.3. Flour preparation

Each *Teff* sample was cleaned manually by sifting and winnowing before milling to remove the damaged grains, stones, dusts, light materials, glumes and stalks and other extraneous materials. Kuncho –white *Teff* (DZ-Cr-387), and Bosete –white *Teff* (DZ-02-161) samples were milled into fine whole flour using disk attrition mill (Brand name and country of origin should be given), with two disks, traditionally used in the cottage *Teff* grain milling house

2.4. Starter culture formulation

For the preparation of a given defined starter culture formulation, a separate pure culture growth of each isolate was separately centrifuged at 5000 g for 5 min at 4º C and. Then pellet was dissolved in 10 mL sterilized distilled water. 3% each of standardized microbes were inoculated in duplicate to 1kg *Teff* flour mixed in 70 mL sterilized distilled water . The *Teff* flour was sterilized using steam sterilization using lid tighten bowel (plastic jar) at 121^{o} C for 20min. 3% of formulated starter culture was added separately in duplicate to the mixed of flour and sterilized water was added to mixing thoroughly until homogenous slurry is obtained. The batter was equally distributed in 6 plastic jars .

2.5. Preparation of *Teff* dough

A 1kg from Kuncho –white *Teff* (DZ-Cr-387), and Bosete –white *Teff* (DZ-02-161) flours was mixed with sterile municipal water in ratio of 1:2 (w/w) as was previously prepared (Zewdu and Abate,2012). A 3% of starter culture '*ersho*' collected from a household and the formulated starter culture was added in different sic plastic jars.(Fig.4A) The plastic jars were used for mixing their contents. After the primary fermentation of each treatment, the supernatant liquid which was slightly yellowish watery content on the surface of fermented batter was decanted. For *absit* preparation, a separate content from each treatment of 200 mL of the fermented mixture was taken aseptically from the container into the saucepan and mixed with 400 mL of water and boiled on a hot plate with a continues stirring for about 5 min. The cooled separate *absit* (below 50°C) from each treatment was added to the main respective part of fermented batter and thoroughly mixed with stirring as indicated in Zewdu and Abate (2012).

Intense fermentation for 2 hours with a noisy air bubbles indicates the second phases of fermentation (Desiye and Abegaz, 2013).Both Bosete *Teff* and Kuncho *Teff* flour (B,K) were used for each of the three treatments (T1,T2,T3) . The resultant dough from each treatment was allowed to ferment for a time interval of 0 hr., 12 hr., and 24 hr. , 36hr., 48hr., 60hr. and 72 hr.

- Treatment 1 - *Teff* flour + 3% Traditional fermented starter culture (*ersho*) from household (T1B,T1K)
- Treatment 2 - *Teff* flour + 3% Formulated starter culture "*ersho*" in normal environment condition (T2B,T2K)
- Treatment 3 - *Teff* flour + 3% Formulated starter culture *"ersho"* in Incubator (T3B,T3K)

2.6. Preparation of *Injera*

Fermented *Teff* batter form each separate treatment was baked by withdrawing 500 mL of fermented dough sample with sterile plastic beaker (jog) and poured out onto the hot clay electric plate, in a circular motion. The baking temperature was between 190 ^{0}C to 210 ^{0}C controlled by checking using thermometer. After 2.5 -3 minutes with steam cooking *Injera* was removed from the hot clay electrical plate, as indicated in Zewdu and Abate (2012). The *Injera* was baked from each of the three treatments were after they reach the optimum fermentation time in which the formulated starter culture in incubator condition (T3B,T3K) at 24hr. , formulated starter culture in environmental condition (T2B,T2K) at 48hr. and traditional household starter culture (T1B,T1K) at 72hr and cooled to room temperature in holder (mesobe) for further analysis.

2.7. Analytical methods

2.7.1. Determination of pH

The pH values were determined using AOAC,981;12 2001 official method using a digital pH meter (pH- 013 High Accuracy Portable pH Meter. The fermented *Teff* batter pH was determined directly using a glass electrode attached to a pH meter. The *Injera* samples (10 g) were mixed with 100 mL of distilled water, and the pH of the supernatant was immediately measured after decanting into a 250 mL beaker.

2.7.2 Titratable acidity analysis

Total Titratable Acidity The total titratable acidity was determined by titrating 10 mL of fermentation aliquot against 0.1 N NaOH to pH 8.30, using phenolphthalein as indicator. The total acid content of the sample was determined as the percentage of lactic acid as follows:

% Lactic acid = mL of 0.1 molar NaOH x 0.9 x 100 (Katinaa *et al*., 2007)

2.8. Determination of shelf life of *Injera*

Each fermented dough samples (10.0 g pieces from all quarters of the sample) was homogenized in 90.0 mL of sterile 0.1% peptone water to prepare stock solution. Stocks were serially diluted (1:10) to 10^{-5} by adding 0.1 mL of stock solution to 9 mL diluent (0.1% peptone water) in dilution tubes. Then, Potato dextrose agar (PDA, HiMedia) amended with 60 mg/l chloramphenicol plates were prepared for yeast and mold determination. For preparation of PDA plates, about 15 mL sterile PDA medium was poured into plates and solidified. Subsequently, 0.1 mL of aliquot was taken from appropriate dilution factor and spread plated onto potato dextrose agar medium. Inoculated PDA plates were then incubated at 25°C for 5-7days.Visible colonies were counted and expressed as the total yeast and mold in colony forming units per gram (cfu/g) of samples (Kiiyukia, 2003).Counts were done starting from 0 day to every two days incubation, and the samples were checked for the formation of mycelium. Yeast and mold counts were started when mycelium was detected on Injera samples during the incubation period.

2.10. Data Analysis

Complete randomized design (CRD) was used and data were statistically analyzed using analysis of variance (ANOVA) in order to assess the significant differences of dependent variables among samples. A least significant difference (LSD) were used to test the effects of treatments when the F-test was statistically significant at ($P < 0.05$) and Duncan's post hook test was applied to rank the mean values of different treatments as computed by SPSS (version 23.00) software

3. Results

3.1. Changes in pH occurred during *Teff* dough Fermentation

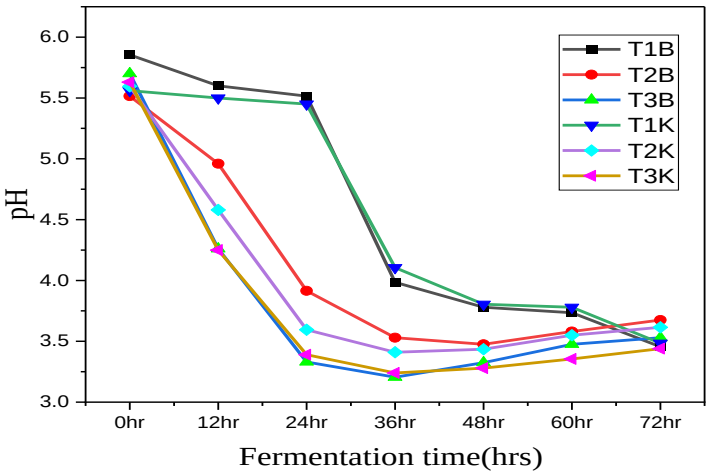

Figure 13: *pH analysis of fermenting Teff doughs from different treatments*

3.2. Changes in titratable acidity value occurred during *Teff* dough fermentation

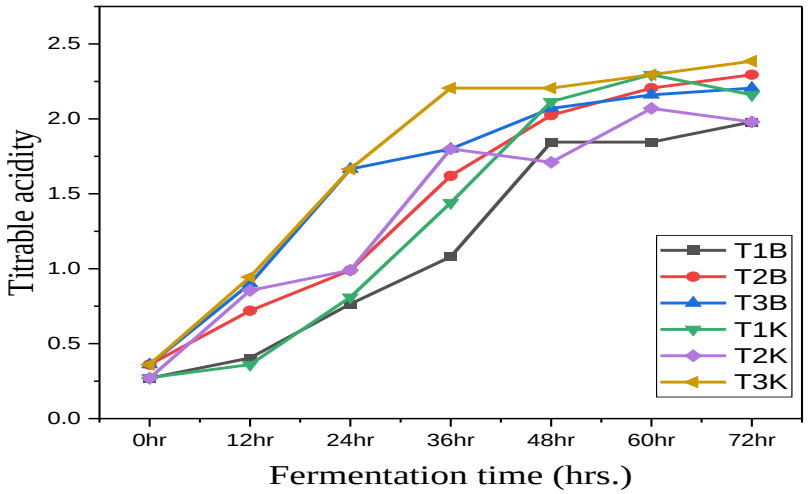

Figure 14:Titritable acidity analysis of fermented Teff doughs from different treatments

3.3. Anti-nutritional composition of the baked *Injera*

Table 1: Anti-nutritional composition data analysis of baked *Injera* from different treatments.

Composition (mg/100g)	Treatments					
	T1B	T2B	T3B	T1K	T2K	T3K
Phytic-acid	246.82±13.870^{b}	236.36±12.61^{e}	248.85±4.62^{a}	241.31±4.290^{d}	231.02±4.35^{f}	243.96±7.50^{c}
Tannin	0.172±0.003^{b}	0.172±0.003^{b}	0.173±0.001^{b}	0.172±0.160^{b}	0.171±0.000^{b}	0.177±0.003^{a}
Oxalate	0.238±0.0446^{b}	0.189 ±0.000^{d}	0.236±0.044^{b}	0.221±0.044^{c}	0.184±0.000^{d}	0.243±0.044^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermenting by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermenting by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

3.4. Mineral Concentration occurred in baked *Injera*

Table 27: Mineral concentration data analysis of baked *Injera* from different treatments.

Mineral content (mg/100g)	Treatments					
	T1K	T1B	T2K	T2B	T3K	T3B
Calcium	197.044±0.000^{c}	231.041±0.114^{b}	242.86±2.22^{a}	242.47±3.68^{a}	115.848± 0.103^{e}	174.288±0.271^{d}
Iron	23.139±0.025^{c}	23.428±0.044^{b}	24.789±0.013^{a}	20.415±0.029^{d}	14.493±0.0424^{f}	16.176±0.034^{e}
Zinc	1.930±0.002^{d}	1.645±0.017^{f}	2.037±0.005^{c}	2.400±0.001^{b}	1.798±0.004^{e}	2.639±0.025^{a}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, T1B;- Bosete *Teff* fermented by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermented by formulated *ersho* in environmental condition, **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition **T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

3.5. Phytic acid to mineral ratio data analysis of baked *Injera*

Table 3: The phytic acid to mineral ratio data analysis of baked *Injera* from different treatments

Phytate: mineral	Treatments					
	T1B	T2B	T3B	T1K	T2K	T3K
Phytate :Ca	0.056±0.004^{d}	0.053±0.005^{d}	0.074 ± 0.006^{b}	0.066± 0.005^{c}	0.054±0.006^{d}	0.106±0.001^{a}
Phytate:Fe	0.734±0.005^{c}	0.763±0.002^{a}	0.660 ± 0.003e	0.740±0.002^{b}	0.695±0.009^{d}	0.647±0.012^{f}
Phytate:Zn	4.880±0.006^{e}	4.951±0.006^{c}	4.988 ± 0.001^{a}	4.883± 0.007^{e}	4.932±0.006^{d}	4.978±0.004^{b}

Note :-Results are presented as means ± SD of twice replications. Mean values with different superscripts in a row show a significant difference ($p < 0.05$).
T1K;- Kuncho *Teff* fermented by traditional *ersho*, **T1B**;- Bosete *Teff* fermenting by traditional *ersho*,T2K;- Kuncho *Teff* fermented formulated *ersho* in environment condition, **T2B**;- Bosete *Teff* fermenting by formulated *ersho* in environmental condition **T3K**:- kuncho *Teff* fermented by formulated *ersho* in incubator condition ,**T3B**;- Bosete *Teff* fermented by formulated *ersho* in incubator condition

3.6. Yeast and mold count of baked *Injera* (cfu/g)

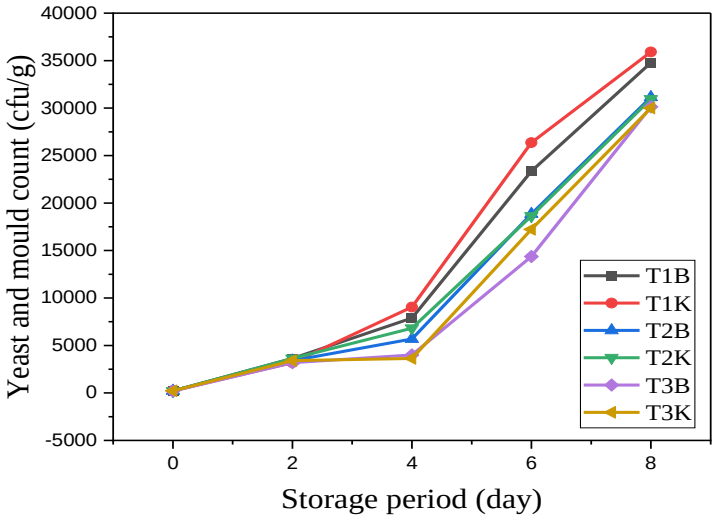

Figure 3 : Colony forming units of yeast and mold/g of *Injera* with respective treatments at different storage intervals.

4. Discussions

The current study presented an evaluation of fermented *Teff* dough on their physicochemical value, anti nutritional composition, mineral bioavailability and Storage duration of *Injera* without visible mold growth. Samples were taken to evaluate the effect of the formulated starter culture in different fermentation condition compare to the traditional starter culture taken from household. Based on this study the average mean value of pH analyzed from fermented *Teff* dough samples had ranged from (3.40-5.85) for bosete *Teff* dough and (3.44-5.59) for kuncho *Teff* dough containing treatments .The highest pH values (5.85, 5.59) fermented for 0 hr. was obtained from T1B and T1K respectively. While the lowest pH values (3.40, 3.44) fermented for 36 hr. was obtained from T3B and T3K respectively (Fig.1).When fermentation time increased the pH value was significantly increased.(Baye *et al*., 2013), (Moges,2021). In different literatures the pH readings of *Injera* was reported with different figures for variable reasons like fermentation time, the removable supernatant liquids remedies and the amount of back slope "*ersho*" used (Yigzaw *et al*., 2004, Urga *et al*., 1997).

The titratable acidity content of the fermented *Teff* doughs with all showed a pattern of increasing from 0 to 72 hr. (Fig.2).The average mean value of titratable acidity analyzed from fermented *Teff* dough samples had ranged from (0.27-2.29%)for bosete *Teff* doughs and(0.27-2.38%) for kuncho *Teff* doughs.(Fig.2).The highest TA value (2.29%, 2.3%) fermented for 72hr. was obtained from T3B and T3K respectively. While the lowest TA value (0.27%, 0.27 %) fermented for 0 hr. was obtained from T3B and T3K respectively. The increase in titratable acidity treatments T2B, T3B, T2K, and T3K was found higher than the treatment T1B and T1K. Similar report showed that increase in titratable acidity has been reported in lactic acid fermentation of various food grains (Rakshit, 2008).

According to the present study, there were significant effect (<0.05) among the treatments on the anti-nutritional composition of baked *Injera* between the treatments samples values. The phytic acid content of the baked *Injera* samples ranged from (216.36 to 238.31mg/100g). The highest calcium content (238.31mg/100g) was obtained from T1B, whereas the lowest calcium content (216.36mg/100g) was from T2B (Table 1).

Phytate degradation had been shown during baking reported in other studies (Fischer *et al*., 2014). Developed starter culture to substantially degrade phytic acid during *Injera* preparation. The Tannin content of the baked *Injera* samples ranged from 0.171to -0.177mg/100g (Table 1).The highest tannin content (0.177mg/100g) was exhibited fromT3K and the lowest tannin content (0.171mg/100g) was obtained from T2K *Injera*. Similar report shows microorganisms somehow reduces tannin content (Woldemariam *et al*., 2019).The oxalate content of the baked *Injera* samples ranged from 0.187to 0.409mg/100g (Table 1). The highest oxalate content (0.409mg/100g) was shown for T1B and the lowest oxalate content (0.187mg/100g) was displayed for T2K (Table 1). This study has similar agreement with studies which show that the use of the processing method such as baking is known to reduce some anti-nutritional factors (Bhandari and Kawabata, 2006). According to the present study, there were slightly significant effect (<0.05) among the treatments on the mineral content of baked *Injera* between the treatments samples values. The calcium content of the baked *Injera* samples ranged from (215.04 to -242.86mg/100g) (Table 2).The highest calcium content (242.86mg/100g) was obtained from T2B and the lowest calcium content (215.04mg/100g) was obtained from T1K (Table 2).According to Yu, *et al.(*2006) report *Teff* is a good source of calcium and minerals as general nutrient composition.

Some other reports also showed that high calcium content may be contributed by high calcium content of *Teff* (195.2 mg/100g) (Bultosa *et al*., 2002).The iron content of the baked *Injera* samples ranged from (20.41 to -24.78mg/100g) (Table 2). The highest iron content (24.78mg/100g) was obtained from T2K and the lowest iron content (20.41mg/100g) was obtained from T1B. This result shows agreement with the previous researchers (USDA ,2015).The total iron contents of all composite *Injera* considered in the study were shown varied from 17.73 to 25.13 mg/100 g (Baye,2014).The zinc content of the baked *Injera* samples ranged from (1.64 to 2.40mg/100g) (Table 2). The highest zinc content (2.04mg/100g) was obtained from T2B and the lowest zinc content (1.64mg/100g) was obtained from T1B.This study was found in agreement with the results indicated by (Abebe *et al*.,2007), and (Baye ,2014). Based on the present study. (T3K, T3B) achieved better mineral content compare to (T1K, T1B).

According to the present study, there were significant effect (<0.05) among the treatments on the phytic acid to mineral ratio of baked *Injera* between the treatments samples values. The ratio value determines bioavailability of the minerals considering their critical ratio limits of phytate: Calcium <0.17 ,phytate:Fe <1 , phytate: Zn <5; as indicated in FAO/WHO (2004). The result showed in (Table 3) the phytate:Ca of the baked *Injera* samples was ranged from (0.05-0.10) The highest (0.10) was shown for T3K and the lowest (0.05) was displayed for T2B.The phytate:Fe of the baked *Injera* samples ranged from (0.69 - 0.94).The highest value (0.94) was obtained from T3K and the lowest value (0.69) was obtained from T2K .The phytate:Zn ratio of the baked *Injera* samples ranged from(4.93 - 4.98. The highest result (4.98) was obtained from T3B and while the lowest (4.93) was obtained from T2K .Such reduction in phytate contents may increase the amount of soluble iron, zinc and calcium and thereby increase their bioavailability (Blandino *et al*., 2003).

The results on the counts of yeasts and molds of baked *Injera* was prepared with the two *Teff* varieties using the formulated starter culture fermenting in incubator condition (T3K,TB), in environmental condition(T2K,T2B) and by using the traditional household starter culture as a control (T1K,T1B). Then stored at a given storage condition for a total of 8 days indicated in (Fig.3).Based on the above result there were significant effect ($p<0.5$) on the bioavailability of minerals between treatments. In which somehow the formulated starter culture higher mineral bioavailability than the traditional starter culture collected from household.

The (Fig.3) showed a pattern in which the counts of the yeasts and molds showed slight growth during the first two days and then after rapidly increased as the storage period continued. The shelf life analysis result of baked *Injera* samples had ranged from ($3.1*10^3$-$4.1*10^4$cfu/g). The highest mold yeast count ($4.3*10^4$ cfu/g) was obtained from TB in day 7. While the lowest mold count result ($3.1*10^3$ cfu/g) was obtained from T3B the first storage day (0 day).

The patterns of rising yeast and mold colony counts were observed to be relatively constant. It is observed that, as exposure to oxygen reduced, the yeast and mold count of *Injera* was also reduced correspondingly. This is due to that, molds are strictly aerobic microorganisms widely spread in nature.

Thus, the limitation of oxygen on their surrounding environment suppresses their growth. For the retardation of mold growth and attainment of long shelf lives, the levels of residual O_2 must be kept below 1% (Guynot *et al*., 2003). In similar study the shelf life of *Injera* was determined by conducting the yeast and mold counts (Girma *et al*., 2013). As displayed on the (Fig.3) the numbers of yeast and mold colony counts of *Injera* at the day of baking for all the treatments were $2*10^1$ cfu/g. As the microbial result shows, the numbers were raised form each baked *Injera* samples fermented by using formulated starter cultures as the number of storage period increased. Relatively, maximum numbers of yeast and mold colonies was observed in the sample which had been fermented by using traditional starter culture. Fungi are the most common spoilers in bakery products.

Commonly a shelf life of 3-4 days may be expected when they are unpreserved (Ryan *et al*., 2008). As shown in the (Fig.3) at ambient storage temperature the maximum yeast and mold count on 7day was observed in the T1B with yeast and mold count of $4.3*10^4$ cfu/g. The colony count for the samples(T1K,T1B) at the day of baking was $2*10^1$ cfu/g which increased from($7.0*10^3$ cfu/g to $2.6*10^4$cfu/g) for T1K and from($7.8*10^3$cfu/g to $2.3*10^4$ cfu/g) for T1B at the third and sixth days of storage period respectively.

Also the maximum number of yeast and mold colony following the control samples were (T2K, T2B) which reached to ($3.2*10^4$ cfu/g , $3.1*10^4$ cfu/g) respectively .As the microbial result shows the minimum yeast and mold count on 7day was observed in the T3B, T2B with yeast and mold count of ($3.1*10^4$ cfu/g, $3.1*10^4$ cfu/g) respectively .In this study the baked *Injera* prepared by using the formulated starter culture (T3B, T3K, T2B, and T2K) had shown better significant effect($p<0.05$) in the mold and yeast counts.

This reduced the number of spoilage microorganism and thereby storage period is higher than *Injera* prepared with the traditional starter culture (T1K,T1B). The shelf life of *Injera* baked using the formulated starter cultures (T2K, T2B, T3K, T3B) was stays for 5 days while shelf life of *Injera* baked by using the traditional starter cultures from house hold stays for 3-4 days. (Fig.3)

According to Zewdu and Abate (2012) studies Penicillium and Rhizopus were more dominant in spoiling *Injera* at lower temperature (16- 200 ^{0}c), while *Aspergillus niger* grow much faster as the temperature gets higher (25-320 ^{0}c). Depending up on the type of mold that dominated the Injera samples at the given condition there might be a variation in the degree of invasion. None of the molds grew when the temperature was kept at 4Oc. (Zewdu and Abate, 2012) whereas, the yeast and mold colony count were vastly reduced due to baking. At the day of baking, yeast and mold count of *Injera* was found to be $2*10^1$ for all treatment samples. (Zewdu and Abate, 2012)

5.Conclusion

According to the data obtained from this study, the results obtained indicated that ($p < 0.05$), there were significant difference on the physicochemical analysis between treatments. In which (T3K,T3B) show lower pH value and higher TA value compare to (T1K,T1B).This result predict that the fermented *Teff* dough using formulated starter culture can reduce the fermentation time by lowering the pH value and in which pH is the effective parameter in the fermentation process. In addition From the study, it was also observed that the fermentation processing methods were lowering the phytate content and improving the bioavailability of essential minerals in *Teff* dough and *Injera* samples ..Generally, from the results of this study, it was observed that formulated starter culture '*'ersho'* were significant effect($P<0.05$) on reduction of the fermentation time, improve consistency , maintaining nutritional value and quality of *Injera* .

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Declarations

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Author Contributions

GD conceived and designed the experiments, collected samples, performed the laboratory works, analyzed the data and drafted the manuscript. BL and HM conceived and designed the experiments, critically comment and revised the manuscript.

Competing interests

The authors declare that there is no competing interest.

Data Availability and materials

All data and materials are within the manuscript.

Ethics approval

The study has got ethical approval by the College of Natural and Computational Sciences Institutional Review Board (IRB), Addis Ababa University.

Consent for publication

This is not applicable.

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