



**WOLKITE UNIVERSITY**  
**COLLEGE OF ENGINEERING AND TECHNOLOGY**  
**DEPARTMENT OF FOOD PROCESS ENGINEERING**

Title: The Impact of Benzoic acid and Clove essential oil on the shelf  
life of Different variety of Teff Injera

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## **List of Abbreviations and Acronyms**

ADI	Average Daily Intake
CSA	Central Statistical Agency (of Ethiopia)
Eh or O/R	Oxidation reduction potential
EHNRI	Ethiopian Health and Nutrition Research Institute
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration (of USA)
ISA	Injera Sucrose Agar
JECFA	Joint Expert Committee on Food Additives
MAP	Modified Atmosphere Packaging
MIC	Minimum inhibitory concentration
PDA	Potato Dextrose Agar
WHO	World Health Organization

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## **Summary**

Injera is staple Ethiopian fermented bread which can be made from different types of cereals, particularly from Teff (*Eragrostis teff*). About two-third of Ethiopian diet consists of Injera and it accounts for about two-third of the daily protein intake of Ethiopian population. It has a very high nutritional value, as it is rich in calcium and iron. However, Injera was usually stay with out spoilage for only three days at ambient temperature and traditional storage conditions, after three days keeping quality was become decreased or spoil as a result of mould. In this study each preservative was added on the Injera dough just immediately before baking. Therefore, selection of appropriate raw materials (teff varieties), preservatives and concentration of preservatives in Injera will be a powerful tool, which leads to a significant inhibition of Injera mould or microbial growth and hence, extension of Injera mould free shelf life can be achieved. The main objective of this thesis will be study the impact of Benzoic acid and Clove essential oil on the shelf life of Different variety of Teff Injera.

**Key words:** Benzoic acid, Clove essential oil, Injera, Teff variety and shelf life.

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# Chapter 1

## 1. Introduction

### 1.1. Background

Teff, *Eragrostis teff*, is believed to have originated in Ethiopia (Vavilov, 1951). Currently, teff is cultivated as a forage crop in countries like Australia, India, Kenya and the Republic of South Africa (Costanza *et al.*, 1979) with limited human consumption in the USA. The crop is the second most widely produced and consumed cereal in Ethiopia next to maize (CSA, 2010/11). Teff has remained an important crop to Ethiopian farmers for several reasons, namely: the price for its grain and straw are higher than other major cereals; the crop performs better than other cereals under moisture stress and waterlogged conditions; its grain can be stored for a long period of time without being attacked by weevils; there is no disease epidemic that has threatened its performance (Ketema, 1989). Teff provides over two-thirds of the human nutrition in Ethiopia, with grain protein content (10-12%) similar to other cereals (Uruga K and HV Narasimha, 1997). Teff proteins have non-gluten nature and owing to prevailing portion of prolamins belong to easily digestible ones which make it a suitable alternative to wheat in the case of celiac disease and gluten-free diet. Besides providing protein and calories, it has high nutritional content, including better amino acid composition, especially lysine, more mineral content (mainly iron, calcium, phosphorus and copper) than other cereal grains, contain B1 vitamin and is rich in fibre (Schneider K and L Anderson, 2010).

The color of teff can vary from white (ivory) to dark brown (black) depending on the variety. In Ethiopia, three major categories can be identified: white (nech), red (quey) and mixed (sergegna). It is also common for wholesalers to further sub-divide white teff into very white (magna) and white (nech). However, given that these classifications are imprecise and subjective, what may be referred as magna by some may be considered as nech by others. White teff generally grows only in the Ethiopian highlands and require relatively good growing conditions. This, along with its higher consumer preference, may justify why white teff is the most expensive type of teff.

However, in recent years, red teff, which is believed to be more nutritious, is also gaining popularity among health conscious consumers in Ethiopia.

Injera is a thin, fermented Ethiopian traditional bread made from flour, water and starter (ersho) which is a fluid saved from previously fermented dough. Tef (*Eragrostis tef* (Zucc) Trotter) is the most popular grain for making injera; although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used. The major quality attribute of a Good injera is its slightly sour flavour (Adamu Zegeye, 1997). Fellow (1997) reported that normal and typical injera is round, soft, spongy and resilient, about 6 mm thick, 60 cm in Diameter with uniformly spaced honeycomb-like 'eyes' on the top. Injera has a very high Nutritional value, as it is rich in calcium and iron. 'Wot' in the Ethiopian national language (Amharic) means a stew which is made from plant and animal products is served with injera. As a result of this, injera is not only a kind of bread it is also an eating utensil (Science of cooking, n.d). Injera is the undisputed national food of Ethiopians (Blandino *et al.*, 2003). According to Ball *et al.*, (1996) about two-third of Ethiopian diet consists of injera and it accounts for about 2/3 of the daily protein intake of Ethiopian population (Arogundade, 2006). Unfortunately, injera storage period does not usually exceed 3 days at ambient temperature under the traditional storage conditions essentially due to mould spoilage.

Food preservation has been a long lasting desire of human beings (Juneja *et al.*, 2008). It was the prerequisite to man settling down in one place, instead of moving from place to place in the never ending hunt for fresh food. The earliest preservation technologies developed were drying, smoking, chilling and heating. Later on, the art of controlling these technologies was developed. The use of various compounds such as salt and spices to preserve foods was also used in ancient times (Zeuthen and Bogh-Sorensen, 2003). Early history is evident of the chemical preservation of food and its various products. The addition of chemicals to food is not a recent innovation; it is being practiced throughout the world (Bohra and Pariah, 2006). Food preservatives are added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes of foods during its shelf life. They also prevent consumer hazards due to the presence of microbial toxins or pathogenic microorganisms and economic losses due to spoilage (Russell and Gould, 2003).

Benzoic acid, sorbic acids, and propionic acid and their respective sodium, potassium and calcium salts are the most commonly used preservatives in foodstuffs. They are generally used to inhibit yeast and mold growth, being also effective against a wide range of bacteria. These compounds are most active in foods of low pH value and essentially ineffective in foods at neutral pH values (Santini et al., 2009). Clove (*Syzygium aromaticum*) is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes. This plant represents one of the richest source of phenolic compounds such as eugenol, eugenol acetate and gallic acid and possess great potential for pharmaceutical, cosmetic, food and agricultural applications. The antioxidant and antimicrobial activity of clove is higher than many fruits, vegetables and other spices and should deserve special attention.

## 1.2. Statement of the Problem

Fungi are the most common spoilers in bakery products, especially injera(fermented bread) and it is responsible for injera spoilage. Consequences of microbial (mould) growth in foods (injera) include endanger of customers as a result of the presence of pathogenic organisms, as well as economic (money, time and energy) losses. Benzoic acid and Sodium benzoate will be a better inhibitor of all fungal species, which prolonged the shelf life of injera and bread (Jay, 2000). As most Ethiopian households usually do not own refrigerators or other instruments to preserve food. Most of the population were live as modernized life style and consumed prepared or ready to eat food. Currently, Injera is exported to North America, Europe and Asia where more Ethiopians are living, However, Injera was usually stay with out spoilage for only three days at ambient temperature and traditional storage conditions, after three days keeping quality become decrease or spoil as a result of mould. Control of shelf life is also important in limiting product and raw material waste by the manufacturer, thus allowing efficient and low-cost food processing. To satisfy consumer demands, reductions in conventionally used scientifically preservation techniques must be accomplished. Antimicrobial agents are chemical compounds added or present in foods that retard microbial growth. Essential oils are naturally occurring compounds and abundant in the environment. Antimicrobs extracted from spices exert directly or indirectly effects to extend foodstuff on microrgasims that spoil the product. Sodium benzoate and essential oil extracted from clove will be used in this research. The over all objective of this research will be study the impact of Clove essential oil and Benzoic acid as preservatives on the extending shelf life of different variety Teff Injera.

### **1.3. Objectives of the Study**

#### **1.3.1. General Objective**

- ❖ To study the impact of Benzoic acid and Clove essential oil on the shelf life of different variety of Teff Injera.

#### **1.3.2. Specific Objectives**

- ✚ To extract essential oil from Clove by using steam distillation.
- ✚ To investigate the effect of teff variety and types of preservatives on the shelf life of Injera.
- ✚ To conduct Microbial analysis.
- ✚ To analyse Sensory acceptability of Injera.

## Chapter 2

### 2. Literature Review

#### 2.1. Teff

Teff is currently the most expensive grain to purchase in Ethiopia as it requires labor-intensive harvesting and processing techniques, and produces especially low yields, thus its availability is limited by high cost (Doris *et al.*, 2010). Although teff covers the greatest space of arable land in Ethiopia, it has the lowest yield per hectare of an average of 910kg. During the 1996-1997 farming season, teff covered 31% of the total landmass, as compared to 17% and 13% for corn and wheat respectively. The total yield for the teff grown in that year was only 26-28% (Doris *et al.*, 2010).

The chemical composition of teff is not far from those of other cereals, nonetheless the micro- and macronutrients level of grain teff is apparently higher than that of barley, wheat and sorghum (Bultosa *et al.*, 2002) and therefore the nutrient composition of grain teff indicates that it has good potential to be used in foods and beverages worldwide. The amino acid composition of grain teff is reported to be comparable to that of egg protein, except for its lower lysine content (Bultosa, 2007). Teff flour has high water absorption capacity which is related to the higher degree of swelling of the teff starches that have a small and uniform granule size, hence, providing larger surface area and thus higher water absorption (Bultosa *et al.*, 2002 and Bultosa, 2007). Teff starch has slow retrogradation tendency (Bultosa *et al.*, 2002), hence, it could have a potentially positive impact on shelf life of baked products. The crude fat of grain teff is around 2%, lower than that of grain maize which is around 4.45%. The typical composition of Protein, fat, ash and carbohydrate contents of teff grain are given as 9.6%, 2.0%, 2.9% and 73.0%, respectively (Patricia and Lisette, 2008).

Teff is a very small grain in which the flour consists of the bran and the germ making teff flour high in nutrient value, as the bran and germ are the most nutritious parts of any grain (Patricia and Lisette, 2008). Teff has very high calcium content, and also contains high levels of phosphorous, iron, copper, aluminum, barium, and thiamin (Patricia and Lisette, 2008). It is considered that teff has an excellent amino acid composition, with lysine levels higher than wheat or barley and slightly

less than rice or oats (Patricia and Lisette, 2008). Teff is high in protein, carbohydrates, and fiber. The protein composition offers an excellent balance among the essential amino acids (Yu, 2006). It contains no gluten so it is appropriate for people with gluten intolerance (Patricia and Lisette, 2008).

## **2.2. Foods based on fermented cereals**

A number of cereal based fermented foods exist in Africa including Kenkey in Ghana, togwa in Tanzania, mawe in Benin and ben-saalga in Burkina Faso (Baye, 2014; Guyot, 2010, and Nout, 2009). In Ethiopia, Injera is the most common and popular fermented food from cereals such as teff, sorghum, maize, millet, and barley. Injera is the undisputed national food of Ethiopians (Chavan and Kadam, 1989 and Oda *et al.*, 1983). Out of these cereals, teff (*Eragrostis teff*) is the major cereal ingredient in Ethiopian Injera (Blandino *et al.*, 2003). Kebede *et al.*, (1984) reported that sorghum ranks second to teff in preference for making Injera. This could be due to the relative brittleness and dryness of sorghum Injera after storage (Adamu Zegeye, 1997). However due to the high cost of fertilizer, engagement of intense labor for cultivation, weeding and harvesting, and the losses during harvesting, the price of teff is generally high (Bekabil *et al.*, 2011) as compared to sorghum and maize so that blending and compositing on the base teff is common in making Injera in spite of the fact that teff has an excellent nutritional value.

## **2.3. Moulds**

### **2.3.1. Food Spoilage by Moulds**

Moulds, those dusty little spots found spreading over bread, cheese, books and other things in the home, cause the loss of millions of dollars to our economy every year and even worse, are menace to our health (Malloch, 1981). There is an increasing knowledge and understanding of the role played by moulds in food spoilage. Especially the discovery of mycotoxin production in foods has highlighted the importance of moulds in food quality. It is, however, only within the last 5 –10 years that major progresses have been made towards the prevention of spoilage caused by moulds. This is due to recent international agreements on taxonomy and analytical methods for food borne moulds, which has led to the discovery, that a specific, very limited funga (= mycobiota) is

responsible for the spoilage of each kind of food. This is called the associated or critical fungi and has been shown to consist of less than ten species (Filtenborg *et al.*, 1996). The microbial spoilage of foods may be viewed simply as an attempt by the food biota to carry out what appears to be their primary role in nature. This should not be taken in the teleological sense. In spite of their simplicity when compared to higher forms, microorganisms are capable of carrying out many complex chemical reactions essential to their perpetuation. To do this, they must obtain nutrients from organic matter, some of which constitutes our food supply (Jay, 2000). Moulds are ubiquitous which can be found in a wide variety of environments, such as in plants, animal products, soil, water and insects. This broad occurrence can be explained by the fact that moulds can utilize a variety of substrates such as pectines and other carbohydrates, organic acids, proteins and lipids. Moreover, moulds are relatively tolerant to low pH, low water activity, low temperature and the presence of preservatives (Huis in't Veld, 1996).

In addition to visible spoilage, moulds can also accumulate toxins hazardous to health. It has now been established that more than 200 different types of moulds do form substances that are orally toxic to man, when growing in certain foods. Although most research has been carried out on the metabolites of *Aspergillus flavus*, it is quite obvious that in addition to the so-called aflatoxins, many other mycotoxins may be of great significance (Huis in't Veld, 1996). Certain moulds are capable of producing toxic and carcinogenic metabolites. Proliferation of these organisms on foods must be regarded as a potential health hazard. A further consequence of such mould growth is economic loss due to poor appearance, off flavours and need for severe trimming (Skirdal and Eklund, 1993)

### **2.3.2. Characteristics of Moulds**

Molds are filamentous fungi that grow in the form of a tangled mass that spreads rapidly and may cover several inches of area in 2 to 3 days. The total of the mass or any large portion of it is referred to as mycelium. Mycelium is composed of branches or filaments referred to as hyphae (Heritage *et al.*, 1996).

Those of greatest importance in foods multiply by ascospores, zygospores, or conidia. The ascospores of some genera are notable for their extreme degrees of heat resistance. One group

forms pycnidia or acervuli (small, flask-shaped, fruiting bodies lined with conidiophores). Arthrospores result from the fragmentation of hyphae in some groups.

There were no radical changes in the systematic of food borne fungi during the 1980s. The most notable changes involve the discovery of the sexual or perfect states of some well-known genera and species. In this regard, the ascomycete state is believed by mycologists to be the more important reproductive state of a fungus, and this state is referred to as the teleomorph. The species name given to a teleomorph takes precedence over that for the anamorph, the imperfect or conidial state. Holomorph indicates that both states are known, but the teleomorph name is used (Jay, 2000).

Some of the genera of moulds that commonly cause spoilage of foods and grains are: *Penicillium*, *Aspergillus*, *Eurotium*, *Fusarium*, *Endomyces*, *Rhizopus*, *Mucor*, *Monilia* and *Cladosporium*.

## **2.4. Factors that affect Microbial Growth on Food**

Fungal growth is influenced by a variety of complex interactions between intrinsic and extrinsic factors (Lopez-Malo *et al.*, 2005).

### **2.4.1. Intrinsic Parameters**

The parameters of plant and animal tissues that are an inherent part of the tissues are referred to as intrinsic parameters (Jay, 2000, Huis in't Veld, 1996). These parameters are Nutrient content, pH, Moisture content, Oxidation-reduction potential (Eh), Antimicrobial constituents and Biological structure.

### **2.4.2. Extrinsic Parameters**

According to Huis in't Veld (1996) and Jay (2000) extrinsic parameters of foods are those properties of the storage environment that affect both the foods and their microorganisms. Those of greatest importance to the welfare of food borne organisms are temperature of storage, relative humidity of environment, presence and concentration of gases and presence and activities of other microorganisms

## **2.5. Methods of Food Preservation**

### **2.5.1. Spoilage and Shelf life of food**

With few exceptions all foods lose quality and potential shelf life at some rate following harvest, slaughter or manufacture in a manner that is very dependent on food type, composition, formulation (for manufactured foods), packaging and storage conditions. Spoilage, or other changes that lead to loss of shelf life, may occur at any of the many stages between the acquisition of raw materials and the eventual consumption of a finished product (Gould, 1996). The shelf-life of a product is defined as the expected time duration that a product will remain organoleptically acceptable. It is a function of holding temperature and the number of microorganisms remaining in it after processing (Doughari et al., 2007).

The principal reactions that lead to spoilage and that are consequently also the principal targets for effective preservation and controls are well known, and relatively few. They include some that are essentially physical, some that are chemical, some that are enzymic and some that are essentially physical, some that are chemical, some that are enzymic and some that are microbiological (Huis in't Veld, 1996). The high increase in food deterioration is due to contamination with micro-organism since the entire environment in which we live is colonized by micro-organisms (Akpan and Kovo, 2005).

### **2.5.2. Preservation of Food**

Food preservation is an action or a method of maintaining food at desirable level of properties or nature for their maximum benefits (Rahman, 2007). All foods begin to spoil as soon as they are harvested or slaughtered. Some spoilage is caused by such microorganisms as bacteria and moulds. Other spoilage resulted from chemical changes within the food itself due to natural processes such as enzyme action or oxidation (Jay, 2000). Preservation is based firstly on the delay or prevention of microbial growth. It must therefore operate through those factors that most effectively influence the growth and survival of microorganisms. Such factors are not numerous. They include a number of essentially physical factors, some predominantly chemical ones and some microbial ones which depend on the nature of the microorganisms that are present. These factors have been categorized

in a number of ways but the most widely quoted categorizations (Huis in't Veld, 1996) separate the major factors into intrinsic factors, and extrinsic factors.

The major food preservation techniques that are employed are therefore all based on a relatively limited set of factors, so that their range is necessarily limited also (Gould, 1996; Jay, 2000). The major food preservation methods are summarized in Table 2.1 below.

Table 2.1. Major methods of preservation

Cold	Heat	Drying	Fermentation	Physical	Chemical
Freezing Chilling	Cooking Pasteurization Canning	Tunnel Solar Spray Vacuum Freeze Drying	Alcoholic Acetic Lactic	Filtration Separation Distillation Irradiation Concentration Modified/controlled atmosphere packing (MAP)	Sugar, alt spices, acid preservatives e.g. benzoates, nitrites Additives e.g. antioxidants

Source (Adapted from Huis in't Veld, 1996)

### A. Food Preservation by Chemicals

According to Jay 2000, the use of chemicals to prevent or delay the spoilage of foods derives in part from the fact that such compounds are used with great success in the treatment of diseases of humans, animals, and plants. This is not to imply that any and all chemotherapeutic compounds can or should be used as food preservatives. Although a large number of chemicals have been described that show potential as food preservatives, only a relatively small number are allowed in food products, due in large part to the strict rules of safety adhered to by the Food and Drug Administration (FDA) and to a lesser extent to the fact that not all compounds that show antimicrobial activity in vitro do so when added to certain foods. Below are described those compounds most widely used, their modes of action where known, and the types of foods in which they are used.

### **i. Benzoic acid / Benzoates**

Benzoic acid and its salt have been widely used in the food industry for many years as important food preservatives in order to inhibit various bacteria, yeasts and fungi growth in acidic media. They are also used in other products, such as pharmaceuticals and cosmetics. As one kind of common chemical, 638,000 tons of benzoic acid is approximately produced globally per year (Qi *et al.*, 2009). According to Jay 2000, sodium benzoate was the first chemical preservative permitted in foods by the FDA in 1908 as a preservative in certain foods and it continues in wide use today in a large number of foods.

### **ii. Sorbic acid / Sorbates**

Sorbic acid ( $\text{CH}=\text{CH}=\text{CH}=\text{CH}-\text{COOH}$ ) is employed as a food preservative, usually as the calcium, sodium, or potassium salt (Jay, 2000 and Santini *et al.*, 2009). Sorbic acid and its potassium salt are the most widely used forms of the compounds and are collectively known as sorbates. Sorbate was first patented by Gooding 1945 as an antifungal agent, and has been used to a growing extent to protect a variety of foods against spoilage by microorganisms (Razavi-Rohani and Griffiths, 1999). Sorbic acid was approved by FDA for use as a food preservative in 1955 (Jay, 2000). Potassium salt is commonly used because it is more stable. Furthermore, its greater solubility extends the use of sorbate to solutions appropriate for dipping and spraying (Gonzalez-Fandos *et al.*, 2007). These compounds are permissible in foods at levels not to exceed 0.2 %. The widest use of sorbates is as fungistats in products such as cheeses, bakery products, fruit juices, beverages, salad dressings, and the like (Jay 2000). The acceptable daily intake (ADI) values, determined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), is 25 mg/kg of body mass for sorbic acid and sorbates salts (Santini *et al.*, 2009).

### **iii. Propionic acid / Propionates**

Propionic acid is a three-carbon organic acid with the structure  $\text{CH}_3\text{CH}_2\text{COOH}$ . This acid and its calcium and sodium salts are permitted in breads, cakes, certain cheese, and other foods (Jay 2000). The antimicrobial effect of propionic acid has been known since 1913. Propionic acids and their salts are primarily inhibitory to molds; however, some species of molds are resistant and can grow

in media containing substantial amounts of propionic acid and its salts (Razavi-Rohani and Griffiths, 1999). They tend to be highly specific against moulds, with the inhibitory action being primarily fungistatic rather than fungicidal. Propionic acid is employed also as a "rope" inhibitor in bread dough (Jay, 2000). Propionic acid has previously been shown to inhibit moulds and *Bacillus* spores, but not yeasts to a large extent, and has therefore been the traditional chemical of choice for bread preservation (Ryan *et al.*, 2008)

## **B. Preserving by Spice Essential oil**

Antimicrobial compounds in plant materials are commonly contained in the essential oil fraction of leaves (rosemary, sage), flowers and flower buds (clove), bulbs (garlic, onion), rhizomes (asafoetida), fruit (pepper, cardamom). According to this ranking, cinnamon and clove were listed as exhibiting a strong inhibitory effect. Conner and Beuchat (1984) screened 32 essential oils from plant sources for inhibitory effects on 13 food spoilage and industrial yeasts and identified cinnamon, allspice, and clove among the most inhibitory tested. Antimicrobial activity of cinnamon, allspice, and cloves is attributed to eugenol (2-methoxy-4-allyl phenol) and cinnamic aldehyde, which are major constituents of the volatile oils of these spices. Allspice contains up to 4.5% volatile oil, of which 80% is eugenol. Clove buds have an average essential oil content of 17% that is 93% to 95% eugenol (Bullerman *et al.*; 1977).

## **Natural Phenolic Compounds**

Derivatives of phenol, called phenolics, contain a phenol molecule with one or more substitutes. This chemical alteration of phenol may increase its antimicrobial activity. Naturally occurring phenolic compounds are widespread in plants and may be found in a great variety of food systems, and as phenol derivatives they may have antimicrobial activity. Concentration of phenolic antioxidants with antimicrobial activity in food products was in the range of 30 to 10,000 ppm, yet these compounds are permitted as antioxidants in concentrations generally up to 200 ppm, based on the fat or oil content of the food product. To comply with U.S. federal regulations, Kabara (1991) suggested the use of phenolic antioxidants in combination with other antimicrobials. Vanillin has antifungal activity (López-Malo *et al.* 1997). Hitokoto *et al.* (1980)

reported that cinnamon bark had the same strong inhibitory effect on the molds *Aspergillus flavus*, *A. parasiticus*, *A. versicolor*, and *A. ochraceus*.

Bullerman *et al.* (1977) reported that cinnamic aldehyde and eugenol, the major constituents of essential oils from cinnamon and clove, respectively, were the active antimicrobial compounds of the oils. Mahmoud (1994) reported that cinnamic aldehyde in lower concentrations than the MIC delayed *Aspergillus flavus* growth for 8 days at 28°C. The presence of 1000 ppm of vanillin inhibited *A. ochraceus* growth for more than 2 months at 25°C in potato dextrose agar (PDA), whereas growth of *A. niger*, *A. flavus*, and *A. parasiticus* was inhibited by 1500 ppm. Conner and Beuchat (1984a) found, studying essential oils of plants and spices against several food spoilage yeasts, that each studied yeast responded in a different way, indicating that extracts can present various modes of action or there are diverse yeast metabolism responses to the antimetabolic effect of essential oils.

## **2.6. Background of Injera**

### **2.6.1. Product Description**

Injera is Ethiopian fermented bread made from different types of cereals. It is large, flat, round and uniformly spaced honeycomb-like eyes, each measuring about 60 cm in diameter and the base has a smooth surface. Injera looks whitish cream, reddish –brown or brown depending on the type of cereal flour used (Fellow, 1997). Injera can be made from teff (*Eragrostis teff*), wheat, barley, sorghum, or maize or a combination of some of these cereals. Whenever the soil type and rainfall patterns are suitable for the cultivation of teff, injera from teff is more favoured than from the other cereals (Mogessie Ashenafi, 2006). According to EHNRI (1997) the various types of Injera produced from the different varieties of cereals do not have significant variation in their calorie, moisture, protein, carbohydrate, or phosphorus nutrients. Significant variations are, however, observed in the other nutrients.

According to CSA (2008) report, teff [*Eragrostis tef* (Zucc) Trotter] has the largest share of area (23.42 %, 2.6 million hectares) under cereal cultivation and third (i.e. after maize and wheat) in terms of grain production (18.57 %, 29.9 million quintals) in Ethiopia. Teff grain commands

premium price among other cereals cultivated in Ethiopia. There is a growing interest on teff grain utilizations because of nutritional merits (whole grain); the protein is essentially free of gluten the type found in wheat and it accounts (Arogundade, 2006) for about 2/3 of the daily protein intake in the diet of Ethiopian population. The grain proteins are also presumed easily digestible because prolamins are very small. Teff grain micronutrient is also apparently high, particularly in iron, a result of agronomic practices used in Ethiopia and fermentation on Injera making.

### **2.6.2 Processing of Injera**

Cereal grains had been one of man's earliest sources of food. One way of processing the grains into food is through fermentation (Taiwo, 2009 and Blandino *et al.*, 2003). The preparation of teff Injera consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures. Temperature in the highlands of Ethiopia is generally between 17 and 25<sup>0</sup>c. The only required ingredients are the tef flour and water (Berhanu Abegaz Gashe, 1985). The method of processing of Injera from its raw materials to the final product is summarized by Fellow, (1997) as described below on Table 2.2 It involves preparing and mixing the ingredients to dough, which is fermented and subsequently thinned to a batter. The batter is then poured onto a hot griddle in a thin layer to cook, developing its colour, flavour and texture.

## Chapter 3

### 3. Materials and Methods

#### 3.1. Experimental Site

This study was conducted at Wolkite University College of Engineering and Technology, Department of Food Process Engineering laboratory.

#### 3.2. Sample Collection

The raw material (White teff, Red teff, Sergegna teff and clove) was purchased from wolkite market and transportation of the raw materials was by car from wolkite town to Wolkite University.

##### 3.2.1. Sample Preparation

###### 3.2.1.1. Preparation of Flour from Teff (white, Red and Sergegna)

The Teff grains was cleaned and milled separately by using a small grinding mill. The flour was stored in a closed plastic container at room temperature until it was used for further experiments.

###### 3.2.1.2. Extraction of Essential Oil

Water was required for steam distillation process as the solvent. According to Hesham,*et al*, (2016), about 100 to 150g, was added to the flask via a glass tube and to a condenser to retrieve the oil. Since the product is oil, some of it stuck to the collection jar, during extraction and some was stuck to the separatory funnel during the separation of the oil from water. Volatilized essential oils was boiled at temperature range of 50°C- 100°C for 2hr. The recovered mixture was allowed to settle and the oil was withdrawn (Hesham,*et al*, 2016). The product was collected and separated using separatory funnel, after the steam distillation is processed. The essential oil was settled at the bottom layer of the separatory funnel. Until no oil is left in the separatory funnel, separation was continued for several times. Oil has high viscosity, so some of the oil stuck inside the instruments. The extracted essential oil was stored at dark place. For analysis, about 18 ml of essential oil was required for treating the sample.

### **3.2.1.3. Baking of Injera**

The teff Injera samples were prepared at Food Process Engineering Laboratory in almost the same way as reported by Fellow (1997). Accordingly, the teff flour mixed 1:2 (w/w) with distilled water and 16 % of Irsho by the weight of the flour was kneaded by hand in a bowl in the traditional way. The resultant dough was allowed to ferment for 3 days at ambient temperature. After this primary fermentation, the dough was mixed 1:3 (v/v) with boiling water, and heated for 15min with continuous stirring. The hot cooked dough (absit) was then mixed back into the fermenting dough, and sufficient distilled water was added to make a batter. The batter was left covered for 2 hour of secondary fermentation. Some more distilled water was added to thin down and form the right consistency batter. The preservative was added on the Injera batters at the concentration of 0.1 ml, 0.5 ml, and 1 ml, Benzoic acid just immediately before baking. The concentration of preservatives in breads is 1000 ppm for Benzoic acid (Jay, 2000). These values correspond to 0.5 grams of Benzoic acid per pound of flour. After the baking process, the experimental and control samples was stored in the laboratory at ambient temperature. The process was the same for essential oil from clove at the concentration of 1 ml, 2 ml and 3 ml (Jay, 2000). Finally, about half a liter of batter was poured onto the hot clay griddle in a circular motion from the outside, working towards the center. After 2-3 min of cooking using electric Injera baking equipment ('metad'), it was removed and stored in a basket called "messhob".

Table 3.1. Processing of Injera from Teff

Process	Notes
1. Raw materials	Teff ( <i>Eragrostis teff</i> ) is an indigenous cereal for making Injera.
2. Clean	All impurities are removed by hand and winnowed. Teff is simply winnowed and sifted through a fine sieve.
3. Hull	Optional (Sorghum, barley and wheat are usually dampened and pounded traditionally in a wooden mortar and pestle to remove the bran).
4. Grind	The sifted teff is ground through a stone mill.
5. Mixing and first Fermentation	Mix the teff flour with water and Irsho. That is flour: water: Irsho =1: 2: 0.16 (w/w) or by weight of the flour. Mix very well and leave it to ferment for three days.
6. Thin and heat	Discard the surface water formed on the top of the dough. For every 1kg of original flour, take about 200ml of the fermented mixture and add twice as much water, mix and bring to a boil. It should be cooled to about 46 <sup>0</sup> c or 115 <sup>0</sup> F before it is mixed into the main part of the dough. Thin the main dough by adding water equal to the original weight of the flour.
7. Batter making and second fermentation	Add the absit to the thinned dough and mix very well (known as batter making). Leave the batter for about 30 minute to rise (the second fermentation), before baking commences. A small portion of the batter is saved to serve as a starter (Irsho) for the next batch.
8. Griddle or baking	Injera is griddled by pouring about two-thirds of a litre of the batter onto the hot greased electrical ‘metad’ using circular motion from the outside towards the center. It is cooked in about 2-3 minutes at a temperature of the metad reaching 90-95 <sup>0</sup> c. Rapeseed oil is used to grease the metad between each one.
9. Store	Several layers of Injera can be stored in a ‘messhob’ (traditional straw basket) with a tight cover for three days in a cool, dry, ventilated place

Source (Fellow, 1997)

## Chapter 4

### 4. Result and Discussion

#### 4.1. pH and Moisture content of injera

Treatment	pH	Moisture content	Microbial		
			$10^{-2}$	$10^{-4}$	$10^{-6}$
BA M 0.1	$3.14 \pm 0.0173^{bac}$	$14.1 \pm 0.461^k$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
BA M 0.5	$3.097 \pm 0.015^{bc}$	$14.2 \pm 0.608^k$	$0 \pm 0^d$	$16.67 \pm 18.86$	$0 \pm 0^d$
BA M 1	$3.107 \pm 0.015^{bac}$	$13.46 \pm 0.7119^k$	$0 \pm 0^d$	$0 \pm 0^c$	$28 \pm 3^c$
BA R 0.1	$3.19 \pm 0.225^{bac}$	$36.09 \pm 0.7068^{bcd}$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
BA R 0.5	$3.12 \pm 0.0173^{bac}$	$29.89 \pm 0.4636^g$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
BA R 1	$3.057 \pm 0.047$	$38.02 \pm 0.807^a$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
BA W 0.1	$3.253 \pm 0.215^a$	$34.17 \pm 0.425^{fe}$	$81.67 \pm 7.23^a$	$0 \pm 0^c$	$39 \pm 3^b$
BA W 0.5	$3.14 \pm 0^{bac}$	$35.87 \pm 0.809^{cd}$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
BA W 1	$3.14 \pm 0.086^{bac}$	$30.93 \pm 0.562^g$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CL M 1	$3.093 \pm 0.058^c$	$36.25 \pm 0.678^{bc}$	$0 \pm 0^d$	$42.3 \pm 28.8^c$	$0 \pm 0^d$
CL M 2	$3.243 \pm 0.172^{ba}$	$33.67 \pm 1.084^{fe}$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CL M 3	$3.063 \pm 0.058^a$	$12.97 \pm 1.557^a$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CL R 1	$3.17 \pm 0.116^{bac}$	$34.817 \pm 0.665^{ed}$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CL R 2	$3.11 \pm 0.095^{bac}$	$32.86 \pm 0.525^f$	$0 \pm 0^d$	$27 \pm 1^b$	$50.3 \pm 1.53^a$
CL R 3	$3.077 \pm 0.011^c$	$26.87 \pm 1.157^h$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CL W 1	$3.167 \pm 0.007^{bac}$	$36.44 \pm 0.916^{bc}$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CL W 2	$3.17 \pm 0.265^{bac}$	$38.543 \pm 0.911^a$	$3.31 \pm 4.5^b$	$0 \pm 0^c$	$50.3 \pm 15.31^a$
CL W 3	$3.143 \pm 0.028^{bac}$	$37.47 \pm 1.883^{ba}$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CM	$3.073 \pm 0.055^c$	$19.17 \pm 0.519^j$	$6.67 \pm 11.55^c$	$0 \pm 0^c$	$0 \pm 0^d$
CR	$3.08 \pm 0^c$	$8.343 \pm 0.586^a$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CW	$3.14 \pm 0^{bac}$	$23.05 \pm 0.060^i$	$0 \pm 0^d$	$0 \pm 0^c$	$0 \pm 0^d$
CV	2.875756	3.077557	54.96295	154.6818	43.61631
LSD	0.1484	1.4176	5.1608	2.01808	5.7381

#### **4.1.1. pH of Injera**

Results of this investigation showed that injera was generally acidic irrespective of the type of preservatives added. The pH value of the samples stored at room temperature ranges from 3.06-3.17. The pHs were 3.12-3.14 for the sample with benzoic acid, 3.14-3.17 for the sample with clove. The pH of the control sample without preservatives were 3.07- 3.14. The results indicated no variation in temperature for all the samples. The acidity for the samples containing benzoic acid and clove essential oil are relatively the same, while it slightly increased for the control samples as compared to the others. As compared to moisture content the pH was shown a good ranges as compared to moistures results because the pH measurement was taken as soon as it was baked so that result has been good enough.

The pH values obtained, when compared with the pH of bread which is mostly between 4.7 and 7.4 (FDA, 2007) was quite low. The high acidity of injera could account for the low numbers and few types of organisms isolated, although the isolates have been found to be associated with food spoilage as described by Ryan, et al., (2008). This might implied that the moulds that spoiled injera were only those which could overcome such acidic environment like *Penicillium* and *Aspergillus* species.

#### **4.1.2. Moisture Content of Injera**

The moisture content of the injera samples after two days of baking were ranging from 8.343-38.5%. According to Guynot et al., (2003) even if bakery products are important staple foods in most countries and cultures, mold growth and drying are two problems that limit the shelf life of both high and intermediate moisture bakery products. In this study also the moisture content of injera had decreased significantly when kept for 2 days. As compared to the pH of enjera the M.C decreases slightly because of due to time delay of measuring the M.C after 2days of baked.

According to Ayub et al., (2003), the overall mean moisture content of different kinds of bread made from wheat range from 37-47 % during storage. Injera had a moisture content of 8.343-38.5% that made injera more perishable than most bread. The reason for injera was more perishable than bread could be due to the larger the surface area of injera which made it more accessible by fungal spores to land and grew. According to Fellow (1997), to prevent mould growth the product should be properly cooled and dried before packing. Hence, any surface moisture will encourage contamination and the injera must not be handled with wet hands as surface moisture will facilitate mould growth.

#### 4.2. Shelf Life of Injera

The effect of diferent preservatives on the shelf life of injera was shown in Table 4.2 and it was determined as the day in which mould was visible to the naked eye. The shelf life of injera without preservatives (control) for white, red and mixed teff was 3-4 days while injera containing 0.3 % calcium propionate was 4-5 days depending upon the storage temperature. Shelf life of injera containing 0.1 % benzoic acid and sodium benzoate were the better preservatives of all tested which prolonged the shelf life of injera up to 10 and 12 days respectively. 0.2 % of potassium sorbate and 0.2 % of blend of the four preservatives had similarly prolonged the shelf life of injera 6-8 days at room temperature.

Table 4.2. Shelf life of injera containing preservatives at room temprature

Injera Treatments	Shelf life ( days)
BA M 0.1	
BA M 0.5	
BA M 1	
BA R 0.1	
BA R 0.5	
BA R 1	

BA W 0.1	
BA W 0.5	
BA W 1	
CL M 1	
CL M 2	
CL M 3	
CL R 1	
CL R 2	
CL R 3	
CL W 1	
CL W 2	
CL W 3	
CM	3 – 4
CR	3 – 4
CW	3 – 4

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). Preservatives may be microbicidal and kill the target organisms or they may be microbiostatic in which case they simply prevent them from growing, thus prolonging the shelf life of the product (Jay, 2000). The preservatives used in this study had prolonged the shelf life of injera from just 3-4 days to up to 10 days. In these 10 days injera could be consumed before it loses its acceptability, since the shelf life of a product is the time a product will remain organoleptically acceptable (Doughari, 2007). So the teff varieties did not show the difference between themselves on the shelf life of injera but the concentration of preservative taken at different levels shown difference. In this case clove essential oil shown longer shelf life as compared to benzoic acid.

## CHAPTER 5

### CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Conclusions

Even though injera is one of the most popular Ethiopian indigenous fermented food, it did not receive the scientific attention it deserves for many years as far as preservation concerned. Its storage period does not usually exceed 3 days at room temperature. To the best of our knowledge, there are no previous reports on the use of chemical preservatives and natural preservatives in any of the Ethiopian traditional foods. This study not only identified the moulds that were responsible in spoiling injera but also evaluated the common chemical preservatives and natural preservatives used in the food industry for the control of injera moulds. In the present study three fungal species from *Penicillium*, *Rhizopus* and *Aspergillus* (*Aspergillus niger*) species were found spoiling injera while keeping it at ambient temperature. The first two species were more dominant in spoiling injera as the storage temperature lowers between 16-20°C, while *Aspergillus niger* is more dominant in spoiling injera stored at higher temperatures. The study also showed that the shelf life of injera can be extended by the use of preservatives and storage conditions that will not favour mould outgrowth. Injera sample without preservatives exhibited the highest percentage of moulds from day 1 to 12 days of preservation and consequently had the poorest keeping quality as compared to those with preservatives. Investigation of the antimicrobial activity of the preservatives also revealed that clove essential oil was the most effective of all the preservatives tested in inhibiting mould growth while benzoic acids was least effective of the preservatives tested.

## 5.2 Recommendations

In developing country like Ethiopia which is still under a struggle to be food secured, losing a substantial amount food due to spoilage is a disaster. Applying the result of this study can contribute a lot in saving a significant amount of injera that is lost in every household per every baking cycle. Moreover, prolonging the shelf life of injera can curb the problem faced to export injera to other parts of the world and to the many Diasporas that miss their home country as well as injera. As the study was conducted in welkite university with specific climatic condition, further investigation on the type and kind of moulds that spoil injera on the warmer parts of Ethiopia should be evaluated. Further studies on the sensory attributes of injera with preservatives must be conducted to see if any of the additives has effect on health and nutritional status. Even if, the use of preservatives is an attractive means to diminish spoilage and insure food safety, consumers today are not in favour of additives as preservatives and an urge to reduce the quantities used exists within the bakery industry. Reduction of preservatives to sub-inhibitory levels has nevertheless been shown to stimulate growth of spoilage fungi in some cases. As a result of this, the application of the hurdle technology (combined technology) should be evaluated to minimize the amount of the chemical preservatives and clove essential oil used in injera. So the application of other alternative preservation methods such as:

- Modified atmosphere packaging (MAP),
- Irradiation,
- Pasteurization of the packaged injera
- Natural plant extracts ( also called green chemicals) and
- Bio-preservation [the use of microorganisms and/or their metabolites] to prevent spoilage and extend the shelf life of injera (and other Ethiopian Traditional foods) should also be evaluated.





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