ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL SCIENCE CENTER FOR FOOD SCIENCE AND NUTRITION

IMPROVEMENT OF INJERA SHELF LIFE AND STALING THROUGH VACUUM AND NON-VACUUM POLYETHYLENE PACKAGING: THEIR SYNERGISTIC EFFECT WITH CHEMICAL PRESERVATIVE

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Signature of the Board of Examiners for Approval

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Declaration

This thesis is my original work and has not been submitted as a partial requirement for a degree in any university

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Abinet Terefe
March 2019

The thesis has been submitted for examination with our approval as university advisors.

1. Dr. Ashagrie Zewdu _____
2. Dr. Shimelis Admassu _____

Abstract

Injera is naturally-leavened fermented sourdough-risen flatbread, indigenous to Ethiopia. It is commonly made from tef (*Eragrostis tef*). Injera has nutritional merits, mainly gluten free protein and high calcium and iron content. Unfortunately, injera has a short shelf life of 3-4 days basically due to mould spoilage. Its traditional storage condition favors mold spoilage and quality loss. Use of critical preservation techniques like packaging is ideal for retardation of mold growth. The application of vacuum packaging (VP) and non-vacuum packaging (NP) of injera, with or without preservative added (sodium benzoate), has been studied for 15 days with the aim of determining their effect on the shelf-life and staling of injera. Samples were tested for microbial load analysis, moisture content (MC), pH, and color "L" value (lightness) determination, visible mold sign inspection and sensory quality evaluation. Oxygen exclusion of the packaging methods and antimicrobial activities of preservative used, prolong the storage duration of injera without visible mold growth to more than 15 days; with VP (vacuum packaging), VP+ (vacuum packaging with preservative) and NP+ (non-vacuum packaging with preservative) treatments. Among these, VP+ had least microbial load ($5.3*10^1$ & $9.0*10^1$ bacterial & yeast and mold colony forming unit (cfu)/g respectively). But it was least effective regarding staling as it had least average scoring of MC, pH and L value (60.96%, 3.33 & 45.92 respectively) and sensory acceptability, basically due to crumbling effect of the packaging method used. Beside, NP+ had lower microbial load ($7.5*10^1$ bacterial cfu/g and $9.0*10^1$ yeast and mold cfu/g). Despite VP and VP+, NP+ was relatively effective method regarding sensory acceptability and staling as it had 62.73%, 3.32 & 48.70 average MC, pH and L value respectively. Generally, packaging methods and preservative used found to have a significant effect ($P<0.05$) on microbial load, physico- chemical properties and sensory attributes of injera. Moreover, it was proved that NP+ was the most effective method to improve the shelf life and staling of injera.

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List of abbreviations and acronyms

ANOVA - Analysis of Variance

AOAC - Association of Official Analytical Chemists

AP - Active Packaging

a_w - Water Activity

CFR - Code of Federal Regulation

cfu - Colony forming unit

CRD - Complete randomized design

CIELAB - Commission International d'Eclairage standard for color measurement

Eh or O/R - Oxidation Reduction Potential

FDA - Food and Drug Administration

FSAN - Food Science and Applied Nutrition

GRAS - Generally Regarded As Safe

LDEP – Low density polyethylene

LSD - Least Significance Difference

MA - Modified Atmosphere

MAP - Modified Atmosphere Packaging

MC - Moisture Content

NP - Non-vacuum packaging

PCA – Plate Count Agar

PDA - Potato Dextrose Agar

RH - Relative Humidity

SPSS - Statistical Product and Service Solutions

UV - Ultra Violet

VP - Vacuum Packaging

1. Introduction

1.1 Background

Bakery products are important staple foods in most countries and cultures (Smith and Simpson, 1995). They are flour-based food baked in an oven such as bread, cookies, cakes, pastries, and pies. Bakery products get deteriorated due to physical, chemical and microbiological factors. Major microbiological loss of bakery products is due to mould growth and problems that are associated with it (Saranraj, 2012). Bread and cake are the main ones from the bakery products. Commercially produced and properly handled bread generally lacks sufficient amounts of moisture to allow for the growth of any organisms except molds. One of the most common molds that grow and spoil bread is *Rhizopus stolonifer*, often referred to as the "bread mold." Storage of bread under conditions of low humidity retards mold growth. Also, breads may undergo a type of spoilage known as staling and ropiness (breaking a batch of dough into two parts). Cakes are rarely undergoing bacterial spoilage due to their unusually high concentrations of sugars, which restrict the availability of water. The most common form of spoilage displayed by these products is moldiness. Growth of molds on the surface of cakes is favored by conditions of high humidity (Jay, 2000).

Injera is an East African sourdough-risen flatbread, which served as a national dish in Ethiopia and Eritrea, commonly made from the tiny, round, iron-rich grain, called teff. However, wheat, barley, corn, and/or rice flour are sometimes used to replace some or all of the teff content due to limited production and expense of teff (Nigatu and Gashe, 1997). Flour, water and sourdough starter (ersho), which is a fluid saved from previously fermented dough are the main ingredients. A mixture of ingredients then let to ferment and baked on clay plate. Then it served with a variety of stews (wot) or sometimes salads (Cooking of Science, 2008).

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) (Ashagri and Abate, 2012). This may arise from traditional storage condition which allows the direct contact of factors that are responsible for spoilage and quality loss of a product. These leads to a need of modifying techniques that can

reduce the direct contact of those factors with the product, and one of such critical techniques is packaging (Fellows, 2000).

Food preservation is an action or a method of maintaining food at desirable level of properties or nature for their maximum benefits (Rahman, 2007). Using of different preservation techniques alone has a contribution for food preservation. Whereas, a combination of several preservative methods (i.e., heating, chilling, drying, salting, curing, acidification, oxygen removal, and fermentation) is better to preserve foods from all aspects of microbial stability and safety as well as sensoric and nutritional quality (Leistner and Gould, 2002).

Packaging is one of preservation methods which increase the shelf life of manufactured foods and ensuring food safety (paine, 1991). Among these technologies, vacuum packaging (VP) and modified atmosphere packaging (MAP) are used to prevent products from contamination and evaporative losses and also extend storage life of food products (Parra *et al*., 2010).

Vacuum packaging is one of the most effective ways to increase the shelf life of food products. The preservative effect of VP is due to the creation of an oxygen-deficient environment resulting in a severe or total inhibition of potential spoilage organisms (Narasimha and Sachindra, 2002). By removing air from around the product, the levels of oxygen in the packaging are reduced to non-significant amount (Jay, 2000). The lack of oxygen then delays ability of oxygen-breathing microorganisms to grow and spoil the product. Moreover, vacuum packaging reduces the amount of spoilage due to oxidation (Galic *et al*., 2009). Vacuum packaging protect the contents from environmental influences such as moisture and oxidation processes, the food contained within then retains its quality and freshness for much longer. In addition vacuum packaging can reduce the volume of the packaging (Smith *et al*., 2004).

Using of preservatives, artificial and chemical preservatives is one of food preservation technique that used to increase the shelf life and reduce the spoilage of food products (Rahman, 2007). Mostly artificial preservatives are safe but some have negative and life threatening effects (Mishra *et al*., 2015). There are different chemical preservatives and these include Propionic acid, Sorbic acid, Benzoic acid etc. which are Generally Recognized as Safe (GRAS) and used for preservation of different foods (Jay, 2000). Using of those techniques combining with others

i.e packaging may give a better synergetic effect; resulting to maintain food qualities and extended shelf life.

1.2 Statement of the problem

Injera is the major and frequently consumed Ethiopian food; about two-third of Ethiopian diet consists of injera (Ball *et al*., 1996). Beside this, with increasing interest and awareness of its nutritional merits such as gluten free protein, iron and calcium content as well as its adaptable taste and other benefits, utilization of injera increase with a time.

Unfortunately, the shelf life of injera does not usually exceed three days at ambient temperature. To mitigate this problem, there was a study conducted by Ashagrie and Abate (2012) on improvement of injera shelf life through chemical preservatives. According to this study, the shelf life of injera without preservatives (control) was 3-4 days while injera containing chemical preservatives was 4-12 days. Also there was a reduction in percentage of mould invasion of the samples with preservatives as compared to the sample without preservative which indicate the effectiveness of the chemical preservatives in inhibiting molds responsible for injera spoilage.

Even though the above study scores a good result on improvement of injera shelf life, fear of toxicity (as injera is a staple food, it is consumed repeatedly which forced the dose of chemical preservative added also increase correspondingly and cause toxicity) and demand for organic (additive free) product; were questionable aspects. Dryness of the product was also another problem. These may raise the necessity of other preservation techniques to replace it as well as use it together with other methods in order to have a better synergetic effect.

The data in most literatures are generally in agreement that, the spoilage of bakery products is mainly caused by aerobic mold growth (Rodriguez, 2000; Sourki *et al*., 2010; Sen *et al*., 2012). The same is true for Injer (Ashagrie and Abate, 2012). This may be due to the traditional storage condition which is suitable for the dominance of oxygen to the storage environment. Hence, packaging methods such as vacuum and non-vacuum poly ethylene packaging may use as an effective preservation techniques, as they are used for exclusion of oxygen and moisture (Brody *et al*., 2001). There are also additional reasons for choice of this preservation technique (packaging), which are the non-applicability of common preservation techniques i.e

pasteurization or sterilization for bakery products (Vlášek *et al.*, 2013), and need of technology which aids the portability of product for export and distribution.

1.3 Objectives

1.3.1 General objective

- ❖ To improve injera shelf life and staling using vacuum and non-vacuum poly ethylene packaging together with chemical preservative.

1.3.2 Specific objectives

- ✓ To carry out analysis of bacterial and yeast and mold colony count of injera with and without chemical preservative (sodium benzoate), packed under vacuum and non-vacuum poly ethylene packaging
- ✓ To evaluate the moisture content, pH and color of injera with and without chemical preservative (sodium benzoate), packed under vacuum and non-vacuum poly ethylene packaging
- ✓ To undertake a sensory evaluation, in order to distinguish the sensory quality and acceptability of injera with and without chemical preservative (sodium benzoate), packed under vacuum and non-vacuum poly ethylene packaging at the maximum visible mold sign-free stage

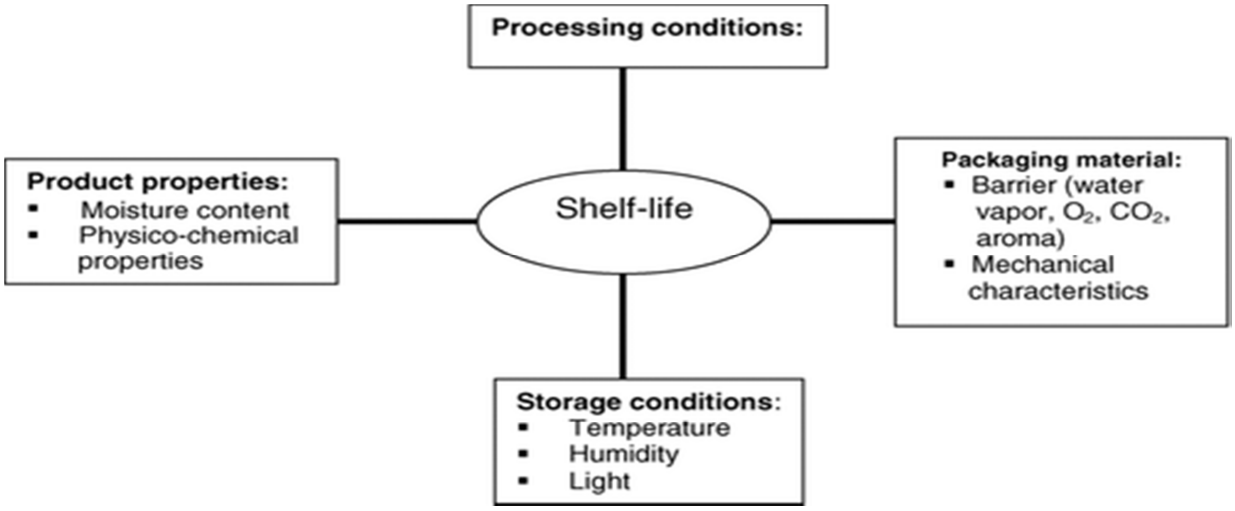
Therefore, this study was conducted to come up with a valuable result by considering positive results and limitations of different studies, and availability of resources. The present work was focused on studying the shelf life and staling of vacuum and non-vacuum packed injera with and without preservative added (sodium benzoate).

2. Literature Review

2.1 Shelf life of food products

Shelf life of a food is the period it will retain an acceptable level of consumable quality from a safety and organoleptic point of view (Galić*et al*., 2009). There are different types of product changes that can limit the shelf life of food. And those changes 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 main factors that cause deterioration of foods during storage are: Spoilage, or other changes that lead to loss of shelf life. According to the Oxford English Dictionary to spoil is to 'deprive of good or effective qualities (Adams and Moss, 2000). A product may become unacceptable as a result of mechanical damage, drying out, discoloration, staling or rancidity which may arise due to; climatic influences (ultra violet (UV) light, moisture vapor, oxygen, and temperature changes), contamination (by micro-organisms, insects or soils), mechanical forces (impact, vibration, compression or abrasion), pilferage, tampering or adulteration (Fellows, 2000). Essentially, different factors affecting the shelf-life of a food are generating on four main conditions (Figure 1), namely formulation, processing, packaging, and storage conditions (Galić*et al*., 2009).

Figure 1: Major conditions that factors affecting the shelf life of food are arise
Adapted from (Galic, 2009)



Product properties (formulation) involves the selection of the most appropriate raw materials and functional ingredients in terms of key factors (include the moisture content (or aw), pH, and the addition of microbial preservatives and antioxidants) that will increase the appeal and ensure safety and integrity of the food for its intended shelf-life (Galić*et al*., 2009). Processing subjects the formulated materials and ingredients to conditions those are unfavorable or inhibitory to undesirable deteriorative reactions and promotive to desirable physical and chemical changes thus giving the food product its final form and characteristics. Once the food leaves the processing stage its keeping properties and the extent to which it will retain its intended attributes is a function of the microenvironment i.e gas composition, moisture content, the relative humidity (%RH), mechanical stresses, light, and temperature which are dependent on both packaging and storage conditions (Galić *et al*., 2009).

On the basis of susceptibility of spoilage, foods can be grouped as perishable (spoil quickly, in days), semi perishable (have a relatively long shelf life, few weeks or months), and nonperishable (have a very long shelf life, many months or years) (Ray and Bhunia, 2008). Although there are various physical, chemical and microbiological factors that cause food deterioration or spoilage, the most important factor 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).

The foods that we eat are rarely if ever sterile, they carry microbial associations whose composition depends upon which organisms gain access and how they grow, survive and interact in the food over time. The micro-organisms present will originate from the natural micro flora of the raw material and those organisms introduced in the course of harvesting, slaughter, processing, storage and distribution. The numerical balance of those microorganisms between the various types will be determined by the properties of the food, its storage environment, properties of the organisms themselves and the effects of processing (Adams and Moss, 2000).

2.2 Factors that affect microbial growth on food

Microbial growth is an autocatalytic process: no growth will occur without the presence of at least one viable cell and the rate of growth will increase with the amount of viable biomass

present (Adams and Moss, 2000). As our foods are of plant and/or animal origin, it is worthwhile to consider those characteristics of plant and animal tissues that affect the growth of microorganisms. The plants and animals that serve as food sources have all evolved mechanisms of defense against the invasion and proliferation of microorganisms, and some of these remain in effect in fresh foods. By taking these natural phenomena into account, one can make effective use of each or all in preventing or retarding the growth of pathogenic and spoilage organisms in the products that are derived from them (Jay *et al*., 2005).

The factors that affect microbial growth in foods and consequently the associations that develop also determine the nature of spoilage and any health risks posed (Adams and Moss, 2000). As suggested from a seminal review by Mossel and Ingram (1955); Those factors that affect microbial growth in foods can be divided into four groups (Table 1); physico-chemical properties of the food itself (intrinsic factors); conditions of the storage environment (extrinsic factors); properties and interactions of the micro-organisms present (implicit factors); and processing factors (Adams and Moss, 2000).

Table 1: Factors affecting the growth of microbial associations in food
Adapted from (Adams and Moss, 2000)

Intrinsic Factors	Environmental factors	Implicit factors	Processing factors
Nutrients	Relative humidity	Specific growth rate	Slicing
pH and buffering capacity	Temperature	Mutualism	Washing
Redox potential	Gaseous atmosphere	Antagonism	Packing
Water activity		Commensalism	Irradiation
Antimicrobial constituents			
Antimicrobial structures			

The last group of factors usually exerts their effect: either by changing an intrinsic or extrinsic property (damage antimicrobial structures, increase nutrient availability and redox potential) or by eliminating a proportion of the product micro flora. So now a day, different studies generally divided those factors affecting microbial growth in to two main general categories called;

intrinsic factors and extrinsic factors (as the remaining group of factors subsumed under those factors) (Adams and Moss, 2000).

2.2.1 Intrinsic factors

Intrinsic parameters are properties that exist as part of the food product itself. These parameters are: pH, Moisture content, Oxidation–reduction potential (Eh), Nutrient content, Antimicrobial constituents, and Biological structures. Under a set of conditions, these parameters promote microbiological growth (Jay *et al*., 2005).

2.2.1.1 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). 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 (complex nutritional or cultural requirements for growth) in their relationships to pH than molds and yeasts, with the pathogenic bacteria being the most fastidious (Ray & Bhunia, 2008).

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 latter type is referred to as biological acidity and is displayed by products such as fermented milks, sauerkraut, and pickles (Jay *et al*., 2005).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).

2.2.1.2 Moisture Content

The water requirement of microorganisms is described in terms of the water activity (aw) in the environment. This parameter is defined by the ratio of the water vapor pressure of food substrate to the vapor pressure of pure water at the same temperature: $aw = p/p_o$, where p is the vapor pressure of the solution and p_o is the vapor pressure of the solvent (usually water).The water activity of pure water is 1.00 (Jay, 2000). Most food borne pathogenic bacteria require aw

greater than 0.9, whereas spoilage molds can grow as low as 0.80. The limiting value of water activity for the growth of any microorganism is about 0.6 and below this value the spoilage of foods is not microbiological but may be due to insect damage or chemical reactions such as oxidation (Adams and Moss, 2000).

The addition of solute decreases aw to less than 1.00. When a solute is added (like salt) to water, the water molecules orient themselves on the surface of the solute, and the properties of the solution change dramatically. Therefore, the microbial cell must compete with solute molecules for free water molecules (Adams and Moss, 2000). Preserving foods with drying or desiccation is also one of the oldest methods, which intended a removal or binding of moisture; without which microorganisms do not grow (Jay *et al*., 2005). In addition to use of salt / sugar and Drying, packaging of the product is used to control the loss or uptake of moisture which is the most important factor that control the shelf life of food. Control of moisture exchange is necessary to prevent microbiological or enzymatic spoilage, drying out or softening of the food, condensation on the inside of packages and resulting mould growth (for example in fresh vegetables and bread) (Fellows, 2000).

2.2.1.3 Redox Potential, Eh

An oxidation-reduction (redox) reaction occurs as the result of a transfer of electrons between atoms or molecules (Adams and Moss, 2000). In living cells an ordered sequence of both electron and hydrogen transfer reactions is an essential feature of the electron transport chain and energy generation by oxidative phosphorylation. The tendency of a medium to accept or donate electrons, to oxidize or reduce, is termed as redox potential (O/R potential) which is expressed by the symbol Eh (Adams and Moss, 2000). When an element or compound loses electrons, the substrate is oxidized, whereas a substrate that gains electrons becomes reduced. Oxidation may also be achieved by the addition of oxygen. Microorganisms display varying degrees of sensitivity to the oxidation-reduction potential (O/R or Eh) of their growth medium or environment (Jay, 2000). Aerobic microorganisms require more oxidized environments (more oxygen) versus anaerobic organisms which require more reduced environments (lacking oxygen).

2.2.1.4 Nutrient Content

In order to grow and function normally, the microorganisms of concern in the food industry require: water, source of energy, source of nitrogen, vitamins and related growth factors, and minerals (Jay *et al*., 2005). Micro-organisms can use foods as a source of nutrients and energy. Thus, the concentration of key nutrients can, to some extent, determine the rate of microbial growth (Bohra and Parihar, 2006).

As sources of energy, foodborne microorganisms may utilize sugars, alcohols, and amino acids. Some microorganisms are able to utilize complex carbohydrates such as starches and cellulose as sources of energy by first degrading these compounds to simple sugars. Fats are also used by microorganisms as sources of energy, but these compounds are attacked by a relatively small number of microbes in foods (Jay *et al*., 2005). The inability of an organism to utilize a major component of a food material will limit its growth and put it at a competitive disadvantage compared with those that can (Adams and Moss, 2000).

2.2.1.5 Antimicrobial Constituents

The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances that have been shown to have antimicrobial activity (Jay, 2000). As a line of defense to attack by microorganisms, the product tissues may contain antimicrobial components, local concentration of which often increases as a result of physical damage. Many natural constituents of plant tissues such as pigments, alkaloids and resins have antimicrobial properties. Classes of antimicrobials known collectively as phytoalexins are produced by many plants in response to microbial invasion (Adams and Moss, 2000). Antimicrobial components differ in their spectrum of activity and potency, they are present at varying concentrations in the natural product, and are frequently at levels too low to have any effect (Adams and Moss, 2000).

2.2.1.6 Biological structures

The natural covering of some food sources provides excellent protection against the entry and subsequent damage by spoilage organisms. Examples of such protective structure are testa of seeds, the outer covering of fruits, the shell of nuts, the hide of animals, and the shells of eggs (Jay, 2000). They are usually a composed of macromolecules relatively resistant to degradation and provides an inhospitable environment for micro-organisms by having a low water activity, a shortage of readily available nutrients (Adams and Moss, 2000).

2.2.2 Extrinsic factors

The extrinsic parameters of foods are not substrate dependent. Extrinsic parameters are those properties of the environment (processing and storage) that exist outside of the food product which affect both the foods and their microorganisms (Jay, 2000). 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 (Jay *et al*., 2005).

2.2.2.1 Temperature of storage

Microorganisms, individually and as a group, grow over a very wide range of temperatures. Therefore, it is well to consider the temperature of growth ranges for organisms in order to select the proper temperature for the storage of different types of foods (Jay, 2000). Microbial growth can occur over a temperature range from about -8°C up to 100°C, at atmospheric pressure (Adams and Moss, 2000).Microbial growth is accomplished through enzymatic reactions. As temperature influences enzyme reactions (with every 10°C rise in temperature, the catalytic rate of an enzyme doubles and reduced to half by decreasing the temperature by 10°C), it has an important role in microbial growth in food (Ray and Bhunia, 2008).

Microorganisms important in foods are divided into three groups on the basis of their temperature of growth, each group having an optimum temperature and a temperature range of growth: thermophiles (grow at relatively high temperature), with optimum at 55°C and range 45-70°C; mesophiles (grow at ambient temperature), with optimum at 35°C and range 10--45°C; and psychrophiIes (grow at cold temperature), with optimum at 15°C and range -5 to 20°C (Ray and Bhunia, 2008). When the foods are exposed to temperatures beyond the maximum and minimum temperatures of growth, microbial cells die rapidly at higher temperatures and relatively slowly at lower temperatures. Influence of temperature on microbial growth and viability is important in reducing food spoilage, enhancing safety against pathogens, and in food bioprocessing (Ray and Bhunia, 2008).

2.2.2.2 Relative humidity of environment

The RH of the storage environment is important both from the standpoint of aw within foods and the growth of microorganisms at the surfaces (Jay *et al*., 2005). Relative humidity is essentially a measure of the water activity of the gas phase. When food commodities having a low water

activity are stored in an atmosphere of high relative humidity water will transfer from the gas phase to the food and the foods pick up moisture until equilibrium has been established. Likewise, foods with a high aw lose moisture when placed in an environment of low RH (Adams and Moss, 2000). There is a relationship between RH and temperature that should be borne in mind in selecting proper storage environments for foods. In general, the higher the temperature is, the lower the RH, and vice versa (Jay *et al*., 2005).

2.2.2.3 Presence and concentration of gases

Oxygen comprises 21% of the earth's atmosphere and is the most important gas in contact with food under normal circumstances. Its presence and its influence on redox potential are important determinants of the microbial associations that develop and their rate of growth (Adams and Moss, 2000). Carbon dioxide (CO_2) is the single most important atmospheric gas that is used to control microorganisms in foods. An increase in the proportion of carbon dioxide and/or a reduction in the proportion of oxygen within specified limits maintain the original product quality and extend the product shelf life. This is achieved by: inhibiting bacterial and mould growth, reducing oxidative changes and controlling biochemical and enzymatic activity to slow down senescence and ripening (Fellows, 2000).

CO_2 inhibits microbial activity in two ways: it dissolves in water in the food to form mild carbonic acid and thus lowers the pH of the product; and it has negative effects on enzymic and biochemical activities in cells of both foods and micro-organisms (Dixon and Kell, 1989). However, close control over the degree of atmospheric modification is necessary to prevent physiological disorders in the living tissues and secondary spoilage by anaerobic microorganisms in non-respiring foods (Dixon and Kell, 1989).

2.2.2.4 Presence and activities of other microorganisms

The inhibitory effect of some members of the food microbiota on other microorganisms is well established. Some foodborne organisms produce substances that are either inhibitory or lethal to others. These include antibiotics, bacteriocins, hydrogen peroxide, and organic acids (Jay, 2000). General microbial interference is a phenomenon that refers to general nonspecific inhibition or destruction of one microorganism by other members of the same habitat or environment; the mechanism for this interference is not very clear. Some of the possibilities are: competition for

nutrients; competition for attachment/adhesion sites; unfavorable alteration of the environment and/or combinations of these (Jay *et al*., 2005).

2.3 Preservation of food products

Food preservation is an action or method of designed to maintain foods at desired level of quality (Marshall, 2006). Foods are perishable or deteriorative by nature. All foods begin to spoil as soon as they are harvested or slaughtered. Major processes of food deterioration are caused by environmental factors such as temperature, humidity, oxygen and light which can be reason for several reaction mechanisms that may lead to food deterioration to such an extent that they are either rejected by or harmful to the consumer (Gallagher,2001). Although food spoilage and/or deterioration may arise from various environmental factors or chemical changes within the food itself due to natural processes such as enzyme action or oxidation, the leading causes of food deterioration and spoilage are microorganisms as bacteria, yeasts and molds (Jay, 2000).

Manipulation of the factors affecting microbial behavior is the basis of food preservation. 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. These 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 (Huis, 1996).

Consumers are increasingly demanding mildly preserved food products that have better fresh like qualities. In addition, changes in retail and internationalization of markets have resulted in increased distribution distances, and longer storage times are required (Sanchis *et al*, 2003).The preservation, processing and storage of the food are vital for the continuous supply of foods during seasons and off-seasons. A number of new preservation techniques are being developed to satisfy current demands of economic preservation and consumer satisfaction in safety, nutritional and sensory aspects. Based on the mode of action, major food preservation techniques can be categorized as: slowing down or inhibiting chemical deterioration and microbial growth; directly inactivating bacteria, yeast, moulds and enzymes and avoiding recontamination before and after processing (table 2) (Marshall, 2006).

Table 2: Major food preservation methods based on mode of action

Adapted from (Marshall, 2006)

Inhabitation of microbial growth, chemical and enzymatic deterioration	Inactivation of bacteria, yeast, moulds and enzymes	Avoiding recontamination before and after processing
Low temperatures Freezing temperatures Reduced water activity Acidification Fermentation Antioxidants Surface coating Structure modification Chemical modification Chemical preservatives Packaging Decrease oxygen Increase CO_2 Gas removal	Pasteurization Sterilization Radiation Electrifying High pressure Chemical Preservative	Packaging Cleaning Sanitary treatment

2.3.1 Chemical Preservatives

The addition of chemicals to food is not a recent innovation but has been practiced throughout recorded history. 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 (Jay, 2000). This is not to imply that any and all chemotherapeutic compounds can or should be used as food preservatives. On the other hand, there are some chemicals of value as food preservatives that would be ineffective or too toxic as chemotherapeutic compounds (Jay *et al*., 2005).Preservatives are 'substances capable of inhibiting, retarding or arresting the growth of micro-organisms or of any deterioration resulting from their presence or of masking the evidence of any such deterioration'. They do not therefore include substances which act by inhibiting a chemical reaction which can limit shelf-life, such as the control of rancidity or oxidative discoloration by antioxidants (Adams and Moss, 2000).

The Code of Federal Regulations (CFR) defines a chemical preservative as, "any chemical that tends to prevent or retard deterioration when added to food" (CFR, 1992). 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 (Jay, 2000).

Table 3: Summary of Some GRAS Chemical Food Preservatives

Adapted from (Jay *et al*., 2005)

Preservatives	Tolerance	Organisms Affected	Foods
Propionic acid/propionates	0.32%	Molds	Bread, cakes, some cheeses, rope inhibitor in bread dough
Sorbic acid/sorbates	0.20%	Molds	Hard cheeses, figs, syrups, salad dressings, jellies, cakes
Benzoic acid/benzoates	0.10%	Yeasts and molds	Margarine, pickle relishes, apple cider, soft drinks, tomato catsup, salad dressing
Parabens∗	0.1%†	Yeasts and molds	Bakery products, soft drinks, pickles, salad dressings
SO2/sulfites	200–300 ppm	Insects, microorganisms	Molasses, dried fruits, wine, lemon juice (not to be used for foods recognized as sources of thiamine)
Ethylene/propylene oxides‡	700 ppm	Yeasts, molds, vermin	Fumigant for spices, nuts
Sodium diacetate	0.32%	Molds	Bread
Nisin	1%	Lactics, clostridia	Certain pasteurized cheese spreads
Dehydroacetic acid	65 ppm	Insects Pesticide	on strawberries, squash
Sodium nitrite‡	120 ppm	Clostridia	Meat-curing preparations
Caprylic acid	-	Molds	Cheese wraps
Sodium lactate	Up to 4.8%	Bacteria	Pre-cooked meats
Ethyl formate	15–220 ppm§	Yeasts and molds	Dried fruits, nuts

Note: *GRAS per Section 201[32] (s) of the U.S. Food, Drug, and Cosmetic Act as amended.*

∗Methyl-, propyl-, and heptyl-esters of p-hydroxybenzoic acid.

†Heptyl-ester—12 ppm in beers; 20 ppm in noncarbonated and fruit-based beverages.

‡May be involved in mutagenesis and/or carcinogenesis.

§As formic acid.

2.3.2 Packaging

Packaging is an important part of all food processing operations and with some (for example canning), it is integral to the operation itself. Packaging may be defined in terms of its protective role as in 'packaging is a means of achieving safe delivery of products in sound condition to the final user at a minimum cost' or it can be defined in business terms as 'a techno-economic function for optimizing the costs of delivering goods whilst maximizing sales and profits' (Fellows, 2000). Although there are a various functions of packaging: to hold the contents and keep them secure until they are used, to protect against mechanical and environmental hazards, to identify the contents and assist in selling the product (Paine, 1991). The principal function of food packaging is to minimize reactions that affect the stability of the contained products. In most cases, the environmentally present gaseous reactants (water vapor, oxygen) can seriously restrict stability under the usual food storage and distribution conditions (Rizvi and Perdue, 1981). Thus, the rate of transport of such reactants across the partial barrier of the package wall can become the limiting factor in shelf-life (Robertson, 1993).

There have been substantial developments in both materials and packaging systems. The main marketing considerations for a package are: the brand image and style of presentation required for the food, its flexibility to change the size and design of the containers, and the compatibility with methods of handling and distribution. Implies the package should be aesthetically pleasing, have a functional size and shape, retain the food in a convenient form for the customer without leakage, possibly act as a dispenser which opens easily and recloses securely, and be suitable for easy disposal, recycling or re-use (Fellows, 2000).

Now a day, industries demand the use of preservation methods which increases the shelf life of manufactured foods ensuring food safety. In this sense, the food industry has developed different packaging technologies such as active packaging, modified atmosphere packaging and vacuum packaging, in order to extend the shelf life of products (Parra *et al*., 2010). Among these technologies, vacuum packaging and modified atmosphere packaging (MAP) become popular as they used to prevent products from contamination and evaporative losses and also extend storage life (Parra *et al*., 2010).

2.3.2.1 Modified atmosphere packaging

MAP is one of the most accepted methods for extending the shelf-life of perishable and semi-perishable food products by altering the relative proportions of atmospheric gases that surround the produce (Sen*et al*., 2012). It uses to minimize the physiological and microbial decay of perishable produce. Modified atmosphere packaging (MAP) application prolongs the shelf-life of product in three ways. Chemically, it controls biochemical processes of degradation and decrease the oxidation. Microbiologically, it prolongs shelf-life by suppressing the growth of bacteria and molds. Physically, it reduces loss of and stabilizes the value of Aw (Vlášek *et al.*, 2013). Modified atmosphere (MA) refers to any atmosphere different from the normal air (20 to 21% O_2, about 0.03% CO_2, about 78 to 79% Nitrogen and trace quantities of other gases) (Sen *et al*., 2012). MA can be created either by direct gas flushing (active MAP) or by respiration of the enclosed product (passive MAP). In both cases, the permeability of the packaging material is important to maintain atmospheres within desired limits. For respiring products, continuous depletion of O_2 and/or increase of CO_2 and water vapor create MA within the package that is known as passive MAP. In active MAP, gas mixture of desired composition is introduced within the package either after evacuation or by a continuous flow of gas mixture to replace the air (Sen *et al*., 2012).

The optimization of the gas mixture composition is critical to ensure both product quality and safety. The gases normally used for MAP include carbon dioxide, oxygen and nitrogen. These gases are used since they are neither toxic nor dangerous and they are not considered as food additives (Smith and Simpson, 1995). Nitrogen is an inert gas, and is used as a filler gas. Because of its insolubility in water, the presence of nitrogen in MAP food can prevent pack collapse that occurs when high concentrations of carbon dioxide are used. In addition, nitrogen on its own can delay oxidative rancidity in low water activity products (Galić *et al*., 2009).

The most important gas, from a microbiological standpoint is CO_2 (Parra *et al*., 2010). Although CO_2 is not known to be lethal to microorganisms, it has shown both bacteriostatic and fungi static properties and will hinder the growth of certain aerobic organisms (Sourki *et al*, 2010). The mode of action of carbon dioxide to its antimicrobial effect is via; exclusion of oxygen (that slows the growth of aerobic spoilage microorganisms), affect the permeability of the cell membrane and soluble in fat and water to reduce the internal pH of the cell (that influence

certain enzyme systems and metabolic activities) (Smith and Simpson, 1995; Galić *et al*., 2009). The use of higher concentrations of CO_2 above 20% shows significant bacteriostatic and fungi static properties and prevents the growth of aerobic microorganisms (Vlášek *et al.*, 2013) for this reason there is an increasing demand for storage of food products in modified atmospheres, which is most often composed of CO_2 alone or mixtures of CO_2 and N_2 (Sourki *et al*, 2010).

One of the most important factors influencing the antimicrobial effect of CO_2 is packaging film permeability. The success or failure of MAP foods depends on both the O_2 and CO_2 impermeability of packaging materials necessary to maintain the correct gas mixture in the package headspace. In addition, films, used in gas packaging should also have low water vapor transmission rates to prevent moisture loss or moisture gain. Polymers commonly used for the gas packaging of food include polyamide (nylon), polypropylene (PP), PVDC, EVOH, and low and high density polyethylene LDPE/HDPE. If only a short shelf life is desired for a gas packaged bakery product, such as bread, LDPE/HDPE bags are suitable. However, if a longer shelf life is desired, individual polymers are laminated to one another, since all the desired characteristics of a packaging film for MAP applications, i.e., strength, impermeability, and heat seal ability, are seldom found in one polymer. The important attributes of laminated films for MAP foods are high lamination bond strength; consistent and uniform thickness; consistent seal strength; and consistent barrier to O_2 and moisture vapor (Smith *et al*., 2004).

2.3.2.2 Active Packaging

Although MAP is one of the most accepted methods for extending the shelf-life of perishable food products, high capital cost of gas packaging machinery has limited the use of this technology. This gives rise to the concept of active packaging. Active packaging (AP) is also an innovative concept that can be defined as a type of packaging that changes the packaging condition, extending shelf life and improving safety or sensory properties while maintaining food quality (Suppakul*et al*., 2003).

Reduction of oxygen is one of the primary uses of active packaging (Galić *et al*., 2009). Active packaging is a packaging with different substances that can absorb or release a specific gas, control the internal atmosphere within the package. The active system can be an integral part of the package or be a separate component placed inside the package that can work as either

absorbing or releasing system (Sen *et al*., 2012). Unfortunately, this addition to the packaging process adds an increased cost which can only be justified with a higher priced value-added product (Galić *et al*., 2009).

2.3.2.3 Vacuum packaging

Vacuum packaging is another way to increase the shelf life of food products by removing air from around the product. It is a method of packaging that removes air from the package prior to sealing. Vacuum packaging involves evacuating most of the oxygen present in the package to levels less than 1%.This low oxygen concentration will help to prevent the growth of aerobic organisms and reduce the rate of oxidative rancidity (Smith and Simpson, 1996).

Vacuum packaging (VP) may be regarded as a special type of MAP, since part of the normal headspace is withdrawn and leaving an altered initial atmosphere (Gorris and Peppelenbos, 1992). During the vacuum packing process all air is evacuated from the pack. It is then hermetically sealed in order to maintain the vacuum and therefore protect the contents from environmental influences such as moisture and oxidation processes. The levels of oxygen in the packaging are reduced that imped the ability of oxygen-breathing microorganisms to grow and spoil the product. The lack of oxygen also reduces the amount of spoilage due to oxidation (Galić *et al*., 2009). Generally, vacuum packing reduces atmospheric oxygen, limiting the growth of aerobic bacteria or fungi, and preventing the evaporation of volatile components sometimes used to have a tight fit to the contents.

2.4 Bakery products

Cereals and cereal products constitute large portion of food resources and consumed by a large number of people worldwide. Bakery products and cereals provide adequate nutrients and calories required every day (Khanom *et al*., 2016).Bakery products have been an important part of a balanced diet for thousands of years as provide the supply of different essential nutrients such as carbohydrates, proteins, lipids, vitamins and minerals (Deibel and Swanson, 2012), (Saranraj, 2012).

Sales of a variety of bakery products have been increased in the past decades. In several countries in the world, up to 50% of the total required calories are supplied by bread alone (Khanom *et al.*, 2016). Indeed, cereal grains, mixed with water and cooked by fire, may have been our ancestors' first "bread type" product. Today, the production of bread and other bakery products has evolved from a primitive, cottage industry into a large-scale, modern manufacturing industry, generating billions of dollars in revenue and employing thousands of personnel (Smith *et al*., 2004). This sustained growth has been driven by consumer demand for convenient, premium baked goods which are fresh, nutritious, conveniently packaged, and shelf stable. Moreover, this increased demand is being met by various new processing and packaging technologies, including modified atmosphere packaging, a technology that has increased the availability and extended both the shelf life and market area of a wide variety of bakery products. At the same time, there has been an increase in in-store bakeries and a renewed interest in "organic," ethnic, and artisan type bakery products (Smith *et al*., 2004).

Several methods can be used to classify bakery products. Classification can be based on product type, (unsweetened, sweetened, or filled goods) or on their method of leavening, (biological, chemical or unleavened). However, from a technological viewpoint, bakery products can be classified on the basis of their pH, moisture content, and water activity (aw) (Doerrey, 1990). Bakery products can be conveniently classified by pH into three groups: (i) high acid bakery products with pH < 4.6, (ii) low acid bakery products with pH between 4.6 and 7, and (iii) nonacid or alkaline bakery products with pH > 7 also classified bakery products on the basis of their aw as (i) low moisture bakery products with a w < 0.6, (ii) intermediate moisture bakery products with aw between 0.6 and 0.85, and (iii) high moisture bakery products with a w > 0.85 and generally between 0.95 and 0.99 (Smith *et al*., 2004).Furthermore, classification of products on the basis of their pH and aw is helpful in recognizing the spoilage and safety potential of bakery products.

2.4.1 Shelf life of bakery products

Bakery products, like most processed foods, are subject to spoilage. The spoilage problems of bakery products can be physical spoilage (moisture loss, staling), chemical spoilage (rancidity), or microbiological spoilage (yeast, mold, bacterial growth) (Smith *et al*., 2004). The predominant spoilage problem is influenced by inter-related factors, specifically storage temperature, relative

humidity, concentration of preservatives, pH, packaging material and gaseous environment surrounding the product and, most importantly, by the moisture content and aw. While physical and chemical spoilage problems occur in many bakery products, microbiological spoilage is often the major factor limiting the shelf life of high and intermediate moisture bakery products (Smith and Simpson, 1995). Most bacteria require a high aw for growth. Hence bacterial problems are limited to bakery products, since aw of bakery products is normally low enough to prevent growth of bacteria (Ray and Bhunia, 2008). Mold growth is therefore, a perennial problem limiting the shelf life of commercially produced and properly handled intermediate and high moisture bakery products (Jay *et al*., 2005). Generally, mold growth and staling are two problems that limit the shelf lives of both high and intermediate-moisture bakery products (Guynot *et al.*, 2003).

2.4.1.1 Mold spoilage of bakery products

Mold spoilage is common in the bakery industry and in many cases, mold growth determines product shelf life of both high-moisture and intermediate-moisture baked goods (Ray and Bhunia, 2008). Mold spoilage of bakery products is, therefore, of serious economic concern to the bakery industry (Smith *et al*., 2004). The common fungal contaminants of bakery products are the species of *Eurotium, Aspergillus,* and *Penicillium*. Other species, such as those of *Cladosporium, Mucor,* and *Rhizopus*, have been found less frequently (Guynot*et al.*, 2003). Since baking destroys most molds, freshly baked products are free of viable vegetative molds and mold spores. However, during cooling and packaging, recontamination can occur and cause growth to take place (Sanchis *et al*, 2003). The products become contaminated as a result of post baking contamination by mold spores from the air, bakery surfaces and equipment, food handlers, and raw ingredients such as glazes, nuts, spices, and sugars (Seiler, 1988). Post-baking contamination is a common problem leading to diminished storage properties of bakery products in many households and bakeries. However, the sources of contamination may be variable and multiple (Nigatu and Gashe, 1997).

Today, the emphasis is given to a prolonging the shelf-life of food. For some food it can be obtained with some operations such as pasteurization or sterilization. However, these methods are inappropriate for bakery products (Vlášek*et al.*, 2013). Rather low temperature and hygienic handling of the material, proper packaging and storage conditions are the prime factors that can

control spoilage of these products (Sen*et al*., 2012).According to (Seiler, 1989),three basic strategies can be used to extend the microbiological shelf life of bakery products: Prevention of post baking contamination by packaging prior to baking or immediately after baking under aseptic conditions, destruction of post baking contaminants on the surface of products after packaging and controlling the growth of post baking contaminants in the packaged products.

While the bakery industry has a wide choice of traditional and novel methods to destroy post-processing mold contaminants, they are not widely used to extend the mold free shelf life of bakery products. The most practical, common, and cost efficient approach used by the bakery industry to achieve this objective is to control the growth of any post-baking contamination in the packaged products. This can be achieved mainly, through reformulation to reduce product a_w, modified atmosphere packaging (MAP) or the use of chemical preservatives (Smith *et al*., 2004).

Mold is an aerobic microorganism, and can be effectively controlled by low residual oxygen levels (less than 1%), better together with carbon dioxide (Black*et al*., 1993). Consequently, MAP becomes one of the best approaches to prevent post-baking contamination. The most common applied levels of CO_2 to N_2, in bakery products are 60:40; however, higher levels of carbon dioxide are sometimes used (Lucas, 2003). While such systems may prevent growth of post baking contamination in the packed products, the major disadvantage of such aseptic packaging conditions is cost (Seiler, 1968).

Using chemical preservatives is another food preservation approach which is most commonly used by the bakery industries. Chemical preservatives used to retard microbiological spoilage by preventing the growth of post-baking contaminant. The most common chemical preservatives used in in bakery products include; calcium and sodium propionate, sorbic acid, potassium sorbate, sodium diacetate, methylparaben, propylparaben, sodium benzoate, and acetic acid(Smith *et al*., 2004).The minimum levels of each preservative required to inhibit the growth of common spoilage microorganisms of concern in bakery products are shown in Table 4.

Table 4: Antimicrobial spectra of preservatives used in bakery products

Adapted from (Smith *et al*., 2004)

Organic acids	Yeasts	Molds	*Enterobacteriacea*	*Micrococcaceae*	*Bacillaceae*
	Minimum levels required for inhibition (% w/w)				
Acetic acid	0.5	0.1	0.05	0.05	0.1
Benzoic acid	0.05	0.1	0.01	0.01	0.02
Citric acid	>0.005	>0.005	>0.005	>0.001	>0.005
Lactic acid	>0.001	>0.002	>0.001	>0.001	>0.003
Propionic acid	0.2	0.05	0.05	0.1	0.1
Sorbic acid	0.02	0.04	0.01	0.02	0.02
Methyl	0.1	0.1	0.2	0.4	0.2
Ethyl	0.1	0.05	0.1	0.1	0.1
Propyl	0.01	0.02	0.1	0.05	0.05

2.4.1.2 Staling

A more serious physical spoilage problem in bakery product is staling. Staling refers to a broad set of sensory and chemical changes that affect the textural properties of the product (Sourki *et al*, 2010). Also it can be defined as "almost any change, short of microbiological spoilage, which occurs in bakery products during the post baking period and making it less acceptable to the consumer" (Hebeda , 1996). Staling, as it is applied to bakery foods, is a generic term covering a number of changes that occur in the products during normal storage. The main components of staling are the physicochemical changes of bread and related products i.e. moisture redistribution, increased firmness, texture deterioration of crumb, loss of crispness of crust and loss of aroma and flavor (Kulp *et al.*, 1981; Quail,1996).

The staling mechanism has been the subject of many investigations. Several studies have suggested that staling is due to moisture migration from the crumb to the crust and, more specifically, from swollen starch to gluten (Kulp *et al.*, 198). Products with higher moisture content, e.g., bread and cakes, stale faster than intermediate or low moisture products, such as cookies or crackers. Staling, however, is not simply due to moisture loss or migration (Kulp*et al*., 1981). It has been shown that the degree and rate of crystallization of starch components, specifically of the non-linear amylopectin fraction, is mainly responsible for staling. Complex formation between starch polymers, lipids, and flour protein is thought to inhibit the aggregation

of amylose and amylopectin (Kulp*et al*, 1981). Thus, the content of these components can influence the rate of staling. Cookies and biscuits, for example, have higher lipid content than bread and tend to stale more slowly. However, these products are more susceptible to lipid oxidation and the development of rancid flavor (Smith *et al*., 2004).

Several commercial methods are used to delay the staling of bakery products, including reformulation with lipids, shortenings, surfactants, emulsifiers, gums, and mono- and diglycerides (Boyle and Hebda, 1990). More recently, an antistaling enzyme has been commercially used to delay staling in many products (Boyle and Hebda, 1990). However, staling can also be delayed through the addition of chemical additives and the use of CO_2enriched atmospheres. For the reason that, when CO_2 dissolved in these sites, the hydrogen bonding between amylopectin branches reduce and this result in less firm and stale product (Smith *et al*., 2004).

2.5 Injera

2.5.1 Product description

"Injera" is an Amharic word for an Ethiopian traditional bakery food product. In Ethiopia, there are many types of traditional fermented foods, amongst which injera is one of the major staple. Injera is unleavened bread usually prepared from fermented cereal, such as tef wheat, barley, maize, sorghum, millet or composite of these cereals. Injera from teff (*Eragrostistef*) is much more (about three times) relished, by most Ethiopians, than that from any other source (Nigatu and Gashe, 1997). This may be due to teff has the largest share of area (23.42%, 2.6 million hectares) under cereal cultivation and third (after maize and wheat) in terms of grain production (18.57%, 29.9 million quintals) in Ethiopia (Ashagrie and Abate, 2012). Teff can be grown in a wide range of soils and under rainfall conditions. Teff grain is tiny with a 1000 kernel weight of about 2 g and it is also highly variable in color, from brown to white but there do not seem to be tannin containing varieties (Emmambux and Taylor, 2013).

Now a day, there is a growing interest in teff grain utilization; this growing interest in the use of teff is primarily derived from its unique nutrient composition. Unlike most cereal grains, teff has a complete set of essential amino acids and is higher in lysine, the most limiting amino acid, than

all other cereals, except oats and rice (Ketema, 1997). The general nutritional composition of teff includes protein 0.2%, fat 0.3%, ash 0.1%, and about 73% starch (Emmambux and Taylor, 2013). Teff has more calcium, copper, zinc, aluminum and barium than winter wheat, barley and sorghum and particularly very high iron (Ketema, 1997). Also teff grain is very rich in iron, as a result of agronomic practices used in Ethiopia. Because of this, the prevalence of iron deficient anaemia among teff injera consumers in Ethiopia is low (Boultosa, 2007). Above all, teff is well known for its gluten free flour which helps to be an alternative grain for celiac (gluten intolerance) patients (Coleman *et al.*, 2013).

Flours lacking in gluten proteins, such as teff, present a challenge to developing a satisfactory baked products due to their inability to rise since it lacks gluten proteins which are imperative to creating volume in baked goods by providing a matrix in which the starch granules can embed (Emmambux and Taylor, 2013). But, this cannot be the challenge for injera since it has a flatten bread like structure which doesn't need to be raised; this enhances injera to be the major baked product produced from teff grain. Thus, utilization of injera also increases correspondingly as of teff utilization.

Injera is a fermented and naturally-leavened flatbread indigenous to Ethiopia. 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 and the base has smooth surface (Attuquayefio, 2014). Injera is elastic despite the absence of gluten, thus is able to roll without cracking.The pliable texture and honeycomb nature of the surface of injera enables it to be used as a utensil in picking up 'wot' (stew made with either vegetables and/or meat). It is commonly served with a variety of stews (wot) or sometimes salads and consumed using a hand as shown on figure 2 (Cooking of Science,2008).

Figure 2: Pictures of injera and its way of serving

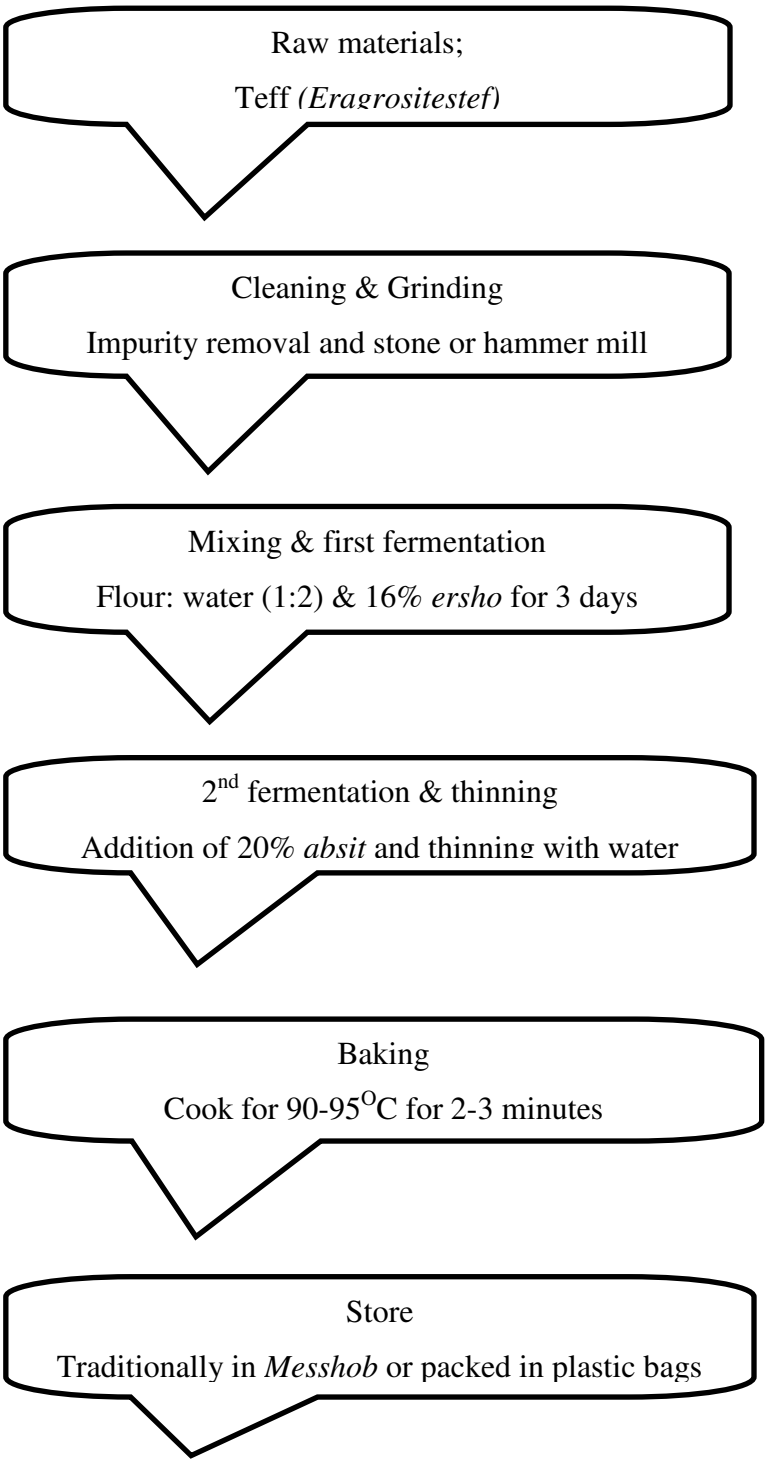
2.5.2 Preparation of Injera

Flour, water and sourdough starter (ersho), are the main ingredients used for preparation of injera. To prepare "injera," teff flour is mixed with water in a container called *bohaka*, which can be a clay, plastic, metal, or wooden container. "Irsho" which is a fermented thin yellowish fluid saved from the previous fermentation is added; then flour, water, and "irsho" are thoroughly mixed by stirring to form a thin, watery paste and left fermentation to occur (Stewart and Getachew, 1962). The preparation of teff injera consists of two stages of natural fermentation, which last for about 24 to 72 hours (Ashagrie and Abate, 2012). The time depends on the altitude of the area, the concentration of "irsho," and the container used (Stewart and Getachew, 1962).

The paste is said to have fermented when gas is produced in considerable quantity in it. After fermentation, the yellow liquid that settles over the paste is poured off and a portion of the fermented paste is mixed and boiled with three part of water to make an additive cooked batter called "A*bsit*". The "absit" is then mixed with the fermented dough and left for about 30 min to rise as of a second fermentation (Ashagrie and Abate, 2012). This aids to get a preferable, clean-looking, fine appearance, soft thin "injera" having an inviting look (Stewart and Getachew, 1962). Finally, baking of injera is usually carried out on a hot earthen plate covered with a metallic or straw lid for about 3-5 min (Nigatu and Gashe, 1997). The baked injera is then keeps well in the traditional storage container called "*mesob*" (a cylindrical container made of grass stems) (figure 3).

Figure 3: Flow diagram for injer preparation from teff

Adapted from (Fellows, 1997)



2.5.3 Quality characteristics and shelf life of injera

Quality 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). As gluten in wheat makes wheat products spongy and elastic, the functional property of proteins in tef is of interest. Apart from proteins, other structural components such as starch, hemicelluloses in tef flour and bacterial exopolysaccharides in fermented teff batter may also contribute to this elastic quality (Attuquayefio, 2014). Although, good quality Injera has a mildly sour taste due to the use of sourdough as a starter for fermentation (Bamforth, 2008),there are also additinal two types of injera called "Aflegna injera" and "Komtata injera" based on the degree of fermentation of the paste from which they are made."Aflegna injera" is thick injera, characterized by its sweet flavor, inviting odor, and rusty red bottom. It is made from relatively unfermented paste 12 to 24 hours old and it does not keep well in the "mesob". Whereas "komtata injera" is made from over-fermented paste and has a very sour-taste.

As a result of the fermentation process, injera is grouped under low pH (high asidic) foods. Although injera is a highly acidic product, its shelf life is not exceed three days at ambient temperature (temperature in the highlands of Ethiopia is between 17 and 25^0C) under the traditional storage conditions because of mold growth (Ashagrie and Abate, 2012). pH of a food has a profound effect on the growth and viability of microbial cells but each species has an optimum and a range of pH for growth. In general, 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).

3 Materials and Methods

3.1 Preparation of Injera

Injera was prepared at home in almost the same way as of the usual house holed preparation, but the proportion of ingredients and timing was modified as reported by (Fellows, 1997). Teff grain (called *nechi* teff) were purchased from local market (wofcho bet), properly sieved for cleaning and milled there. Then, teff flour was send to home for further processes. Teff flour and clean water in the ratio of 1:2 (w/v) and 16 % of starter (ersho) by the weight of flour were mixed in a bowel and kneaded by hand. The mixed ingredients were allowed to ferment for 3 days at ambient temperature. After the primary fermentation, 20% of "Absit" by weight of flour was prepared from the fermented dough itself mixed with boiled water by ratio of 1:3 (v/v) and cooked for 15 minutes with continuous stirring. The cooked batter were left to cool to 45^0c and added back on fermenting dough. Then, a batter were left for about 2 hours to rise as a second fermentation, and Some more water were added to thin down and form the right batter consistency. Finally, about half a litter of the prepared batter were poured and baked for 2-3 minutes on a round shaped electric clay plate which is traditionally called *Mitad*. The baked Injera was taken out from *mitad* and placed in smooth and clean table covered with clean towel with 4 different blocks, each block was having 10 injeras. Then it was let to cool for 30 minutes (after baking of the last injera) under room temperature.

Then after the baking process, the experimental and control samples **(total of 5 treatment samples i.e Non-vacuum packed (NP), Vacuum Packed (VP), Non-vacuum packed with preservative (NP+), Vacuum packed with preservative (VP+) and control (C))** were packed accordingly and sent to AASTU Department of FSAN laboratory and stored there at room temperature for further analysis.

Figure 4: Picture of stored injera samples kept in the laboratory

3.2 Method for addition of chemical preservative (sodium benzoate)

0.1% sodium benzoate by weight of teff flour was added immediately before baking to prevent the chemicals from retarding the second fermentation. Sodium benzoate was chosen based on its effectiveness shown on the previous study by Ashagrie and Abate (2012).

3.3 Pre- packaging activities

Well cooled injera for experimental sample were rolled up and put in a clean and chemically sterile low density poly ethylene bag (LDPE), which is disinfected with 70% alcohol. The process was done by inserting one injera per bag with a hygienic manner and those experimental samples were sent to ZNL enterprise factory for packaging processes. Whereas the control sample were put in a traditional storage container called *Messob* which is covered with clean plastic in the same way for house hold use.

3.4 Method for vacuum and non-vacuum packaging of injera

For vacuum packaging, "HenkoVac Single Chamber Vacuum Sealing Packaging Machine" which was found at ZNL enterprise's injera packaging room was used to pack the samples. The instrument used to create a vacuum and simultaneously seal the pack in hermetic manner. The technical parameters, i.e. evacuating capacity, working length and sealing temperature of the instrument were adjusted on a control board of the instrument based on its manual.

For non-vacuum packaging, a simple sealing instrument was used. And the samples were packed under a normal atmospheric condition since the packing principle of this machine is simply sealing the bag with its content without changing (removing or adding) its air composition.

3.5 Experimentation

From each treatment, one set of a sample were taken for evaluation on every 3 days starting from the day of the packaging. The sampling was done by taking pieces of injera from every quarter of the injera roll and blending all together. The samples were stayed for 15 days for analysis, but each test was terminated when a visible sign of mold was appeared. Testing of all the experiments were performed in duplicate.

3.6 Microbial analysis

3.6.1 Sample processing

Each sample (10.0 g pieces from all quarters of the sample) was homogenized with 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, plate count agar (PCA) plates and Potato dextrose agar (PDA) plates were prepared for bacterial determination and yeast and mold determination respectively. For preparation of PCA plates, 1 ml of diluted sample was dropped on the center of sterile and correctly labeled plate, then about 15 ml molten plat count agar was poured on it and gently rotated by hand for better mixing. Whereas 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 potato dextrose agar media which was prepared in advance. PCA plates and PDA plates were then incubated at 35^0C for 2 days and at 25^0C for 5 days respectively.

3.6.2 Bacterial count

Aerobic Plate Count (APC) method was performed as an indicator of bacterial population on the samples. Plate count agar media was used to determinate the total bacterial count. 1 ml diluted sample were inoculated on PCA medium using pour plate technique and PCA plates were

incubated at 35°C for 48 hours. The colonies were then counted and expressed as colony forming units per gram (cfu/g) of samples (Kiiyukia, 2003).

3.6.3 Yeast and Mold count

Diluted sample (0.1 ml) were inoculated on to potato dextrose agar (PDA) medium supplmented 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. Visible colonies were counted and expressed as the total yeast and mold in colony forming units per gram (cfu/g) of samples (Kiiyukia, 2003). After two days' incubation, samples were checked for the formation of mycelium. Where mycelium was detected, readings were taken at an earlier stage from any of the dishes used in the count.

3.7 Physico-chemical analysis

3.7.1 Moisture content determination

The moisture content (MC) of the samples was determined by oven drying method. 5g of portion of the sample (well homogenized sample taken from all the quarters) were dried in hot air oven at 105^{o}C for three hours and the drying was proceeding until constant weight obtained (Gaithersburg, 2000). Moisture content was then calculated as:

$$\% \text{ Moisture content} = \frac{\text{M initial} - \text{M final}}{\text{M initial}} * 100\%$$

3.7.2 pH determination

The pH of the samples was measured using a digital pH meter (pH- 013 High Accuracy Portable pH Meter). The pH meter was calibrated with standard buffering solutions at pH 4 and 7, and then each injera suspension (a well homogenized mixture of 10g of ground injera with 100 ml distilled water) was measured (Parra *et al*., 2010).

3.7.3 Color determination

The samples for color determination were taken to AAU department of chemical and Biological engineering instrumental laboratory and the color were determined by electronic Spectrophotometer (Konica Minolita Cm-600d Spectrophotometer) which is handheld, portable measurement instrument designed to evaluate the color of various samples. A small portion from each samples were detected by the instrument after calibration with its whitish and darken color

standard. The results were displayed in terms of CIELAB color space (L* a* b* value), which is an international standard for color measurements adopted by the Commission International d'Eclairage (CIE) in 1976. This standard expresses color as three numerical values, L* is the luminance or lightness component, which ranges from 0 to 100, and parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components, which range from –120 to 120 (Markovic*et al*, 2013).

3.8 Determination of storage duration of Injera without visible mold growth

Inspections for visible mold growth on the samples were performed at every three days interval, starting from the day of packaging. The storage duration were considered and recorded as the time period from packaging to the day in advance of observation of visible mold growth (Delnobile*et al*., 2003).

3.9 Sensory evaluation

The samples for sensory analysis were prepared and packed as the same way done previously for other analysis, except baked on different days. This was aimed to fit the predetermined maximum shelf stable stage of the samples to the date of sensory analysis. Hence, samples for VP, NP+ and VP+ treatments were prepared 15 days in advance of date of sensory analysis and samples for NP treatment and control were prepared 7 days and 4 days in advance respectively.

A descriptive sensory analysis was performed using 12 semi trained panelists (Heymann et al., 2012); from students and staffs of AASTU, who has knowledge about sensory quality of injera since they regularly consume it as their staple food. 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.

3.10 Experimental design and statistical 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

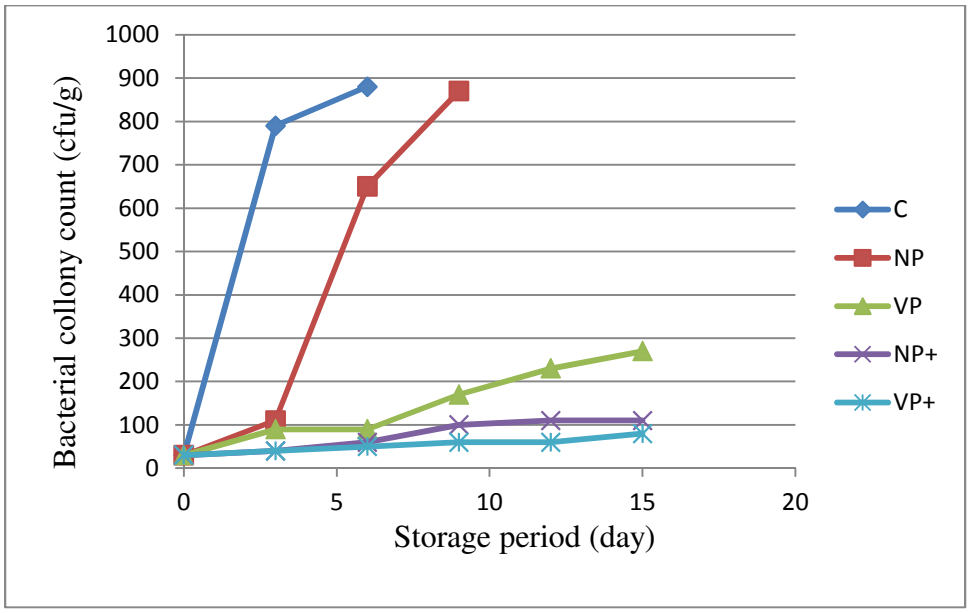
when the F-test was statistically significant at $p<0.05$ and Duncan's posthok test was applied to rank the mean values of different treatments as computed by SPSS (version 20.00) software.

4 Results and Discussion

4.1 Bacterial colony count on injera

The results on the colony count of bacteria on injera with different treatments at a given storage intervals are shown in figure5. The graph shows the patterns of bacterial exposure of injera, as determined by the agar plate technique.

Figure 5: Colony forming units of bacteria/g of injera with respective treatments at different storage intervals.



For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage respectively since the samples were spoiled.

Treatments had significantly ($p<0.05$) affected the bacterial count of injera. As showed on the graph, the numbers of bacterial colony counts/g of injera at the day of baking for all the treatments were $3*10^1$ cfu/g. And it was raised for all treatments, as the number of storage period increased. These increasing patterns were vastly observed on the first two treatments (injera containing no preservative and not packed (C) and non-vacuum packed injrera without preservative (NP)). Relatively maximum number of bacterial colonies were observed in injera containing no preservative and not packed (C). There were $3*10^1$ cfu/g at the day of baking which was increased to $7.9*10^2$ and $8.8*10^2$ cfu/g of injera at third and sixth day storage periods respectively. This higher bacterial colony count among the treatments may come from the effect

of post baking contamination. Since the unpacked injer doesn't have a protection from exposure to external environment, it had a higher possibility for environmental contamination. Additionally, the exposure to environmental oxygen was also another factor which aids the bacterial growth.

Likewise non vacuum packed injrera without preservative (NP) shows higher number of bacterial colonies following the control one. Its number of bacterial colony forming units was raised from $3*10^1$ to $8.7*10^2$ cfu/g on the first to the ninth day of storage respectively. As this treatment was packed, the sample was protected from environmental exposure which reduces the possibility of post baking contamination. However, as packaging method used relatively allows head space oxygen, it played a positive role to support bacterial growth on enjera samples packed under this treatment (NP).

In case of non-vacuum Packed and vacuum packed injera with preservative sodium benzoate (NP+ and VP+), the increment of colonies count were not much across a period. The first counts of colonies were $3*10^1$ for both NP+ and VP+ treatments which increased to 1.1×10^2 and 8×10^1 respectively at 15^{th} day of storage. Whereas the increment of bacterial colonies count for vacuum packed injera without preservative (VP) was found to be higher than NP+ and VP+ samples, which was reached to 2.7×10^2 at the 15^{th} day of storage. Thus, VP+ was proved to be most effective treatment against bacterial spoilage followed by NP+. This indicated that applying a chemical preservative (sodium benzoate) as well as using vacuum packaging method gave a better control against bacterial inhibition.

The addition of chemical preservative sodium benzoate also had impact on a bacterial colony count of pre baked batter. The bacterial colony counts of injer batter immediately before baking were $2.43*10^5$ and $2.66*10^5$ for a batter with and without preservative sodium benzoate respectively. This indicates the preservative had a bit reduction effect on bacterial colony count, even in a short period of its application and its impact may increase with increase in exposure time. Whereas the bacterial colony count were vastly reduced due to baking and the bacterial colony count of injera at the day of baking was found to be $3*10^1$ for all treatments.

This study has revealed presence of microorganisms in injera packed under two different packaging methods with and without preservative sodium benzoate. There is no study performed on injera preservation via different packaging methods such as vacuum and modified gas composition packaging methods. However, the results of this study are supported by a lot of studies have been done for the preservation of bakery products as bread (Sourki *et al*, 2010; Vlášek *et al.*, 2013; Roderiguez*et al*, 2000; Black *et al*, 1993; Parry, 2012). For example, as Sourki *et al* (2010), bread slices packed under CO_2/N_2 shows significant bacteriostatic and fungi static properties. This shows exclusion of oxygen in one or other way has a great role in preventing the growth of aerobic microorganisms. Whereas, the microbial load of air and relative humidity inside the packing played a major role on a growth of microorganisms. Also the packaging film may give a rather wide range of various oxygen and water vapor transmission rates; these variables were considered to have a possible effect on numbers of bacteria in the product (Baran*et al*.,1970).

The use of preservative had also a positive result, as sodium benzoate has long been used as an antimicrobial additive for foods. The sodium salt is preferred because of the low aqueous solubility of the free acid. In use, the salt is converted to the acid, the active form. Sodium benzoate is generally considered to be most active against yeasts and bacteria (El-Shenawy and Marth, 1988).

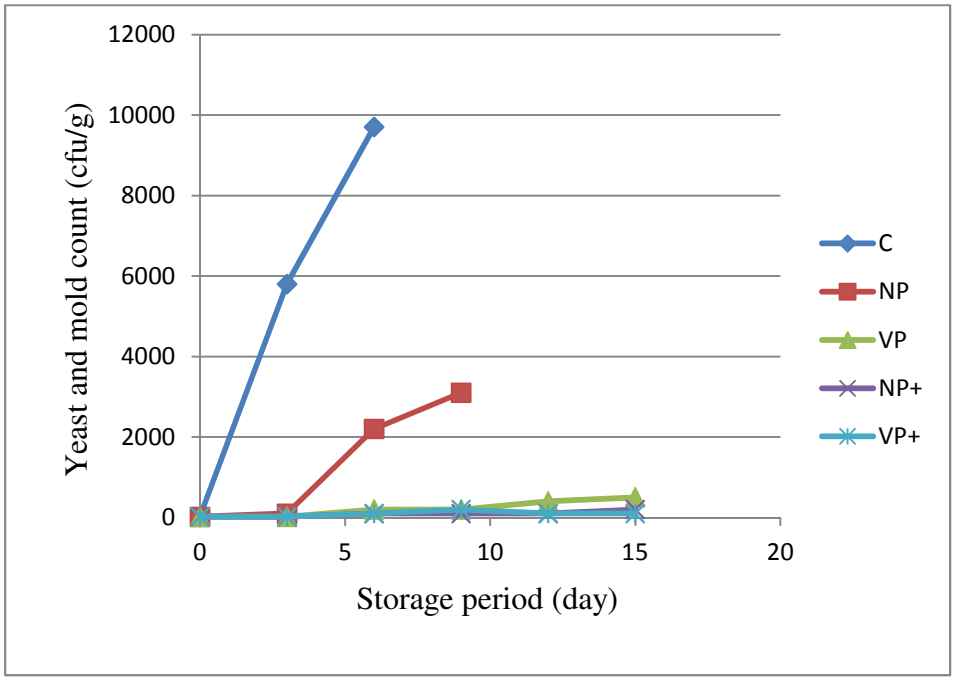
Since there are no available previous findings that deal with microbial loads of injera, the results of this study are compared with some relatively closer findings. According to Khanom *et al* (2016), Average counts of total hetrophilic bacteria in unpacked and packed bread (breads packed with different methods, which are commercially available at supermarkets) sample were 2.01×10^6 and 3.30×10^6 respectively. The result was much higher than the average bacterial colony counts of this study ($5.66*10^2$ and $2.54*10^2$ for unpacked and differently packed injera samples respectively). This may be due to the pH value difference, as injera has pH less than 4.5 (acidc range which is not suitable for bacterial growth) whereas bread has pH near to neutral. International microbiological standards recommended units of bacterial counts for dry and ready to eat foods are $<10^3$cfu/g for total heterotrophic bacteria (Daniyan and Nwokwu, 2011). Total heterotrophic counts indicate general microbiological quality and hygienic status of any food sample. However, in foods with a pH below 4.5 pathogens would not be expected to survive; the

organisms present would be limited to yeasts, molds and a few acid tolerant bacteria (Ray and Bhunia, 2008).

4.2 Viable yeast and mold colony count on injera

The results on the colony count of yeast and mold on injera with different treatments at a given storage intervals are shown in figure 6. The graph shows the patterns of yeast and mold count of injera across storage period.

Figure 6: Colony forming units of yeast and mold/g of injera with respective treatments at different storage intervals.



For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage respectively since the samples were spoiled.

According to the results, treatments had significantly ($p<0.05$) affected the yeast and mold colony count of injera. As displayed on the graph, the numbers of yeast and mold colony counts/g of injera at the day of baking for all the treatments were $2*10^1$cfu/g. And the numbers were raised for all treatments, as the number of storage period increased. While the increment of yeast and mold colony count were vast for the first two treatments (injera containing no preservative and not packed (C) and non-vacuum packed injrera without preservative (NP)).

Relatively, maximum numbers of yeast and mold colonies was observed in the control sample followed by NP sample. The colony count for the control sample at the day of baking was $2 *10^1$ cfu/g which increased to $5.8*10^3$ and $9.7*10^3$cfu/g at the third and sixth days of storage period respectively. Also the maximum number of yeast and mold colony for NP samples were reached to $3.1*10^3$cfu/g which is highest number following the control sample. This higher increment of yeast and mold colony count were arised from the effect of post baking contamination and availability of accessible oxygen which is ideal to facilitate yeast and mold growth on injera samples.

Whereas the maximum number of yeast and mold colony counts appeared on the remaining three treatments (VP, NP+ and VP+) were relatively smaller; $5*10^2$, $^2*10^2$ and $1*10^2$ respectively. And the yeast and mold colony count increasing patterns were looked relatively constant. This indicated that, vacuum packing gave a better inhibitory effect on yeast and mold growth on injera. Beside these, applying a chemical preservative (sodium benzoate) gave a superior synergetic effect on yeast and mold growth retardation. Thus Vacuum Packed injere with preservative sodium benzoate (VP+) as well as non-vacuum packed injera with preservative sodium benzoate (NP+) were proved to be most effective treatments against yeast and mold growth.

The addition of chemical preservative sodium benzoate also had impact on yeast and mold colony count of pre baked batter. The yeast colony counts of injera batter immediately before baking were $1.51*10^5$ and $3.06*10^5$ for a batter with and without preservative sodium benzoate respectively. This indicates the preservative had a positive result on yeast and mold colony count reduction. This reduction effect may increase with increase in time of exposure. 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.

In this study the effect of packaging and chemical preservative (sodium benzoate) on the total yeast and mold colonies count of injera was evaluated. The result obtained were corroborated those from other related studies, which shows using preservatives as well as eliminated O_2 amount has significant mold growth inhibitory effect. According to Khanom *et al* (2016), the total yeast and mold count of packed (different packed bread collected from the supermarket)

and unpacked bread samples were evaluated. And the result showed that unpacked bread samples had a bit higher yeast and mold count than packed bread samples which was $1.01*10^5$ and $1.05*10^5$cfu/g respectively. Also as reported by Rodriguez (2000), the maximum total yeast and mold count of bread packed with normal atmospheric air was 5.98 log cfu/g, which is much higher than the maximum score of yeast and mold count of bread samples packed under absence of oxygen (replaced by different proportion of other gases), which was 2.00 log cfu/g.

As the above findings implies reduction of oxygen exposure or packaging of bread samples has a positive result on reducing of yeast and mold count on the samples. As found in the present study, the same is true for injera. The maximum yeast and mold count of unpacked, non-vacuum packed and vacuum packed injera were $9.7 *10^3$, $3.1*10^3$ and $5*10^2$ respectively. 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).

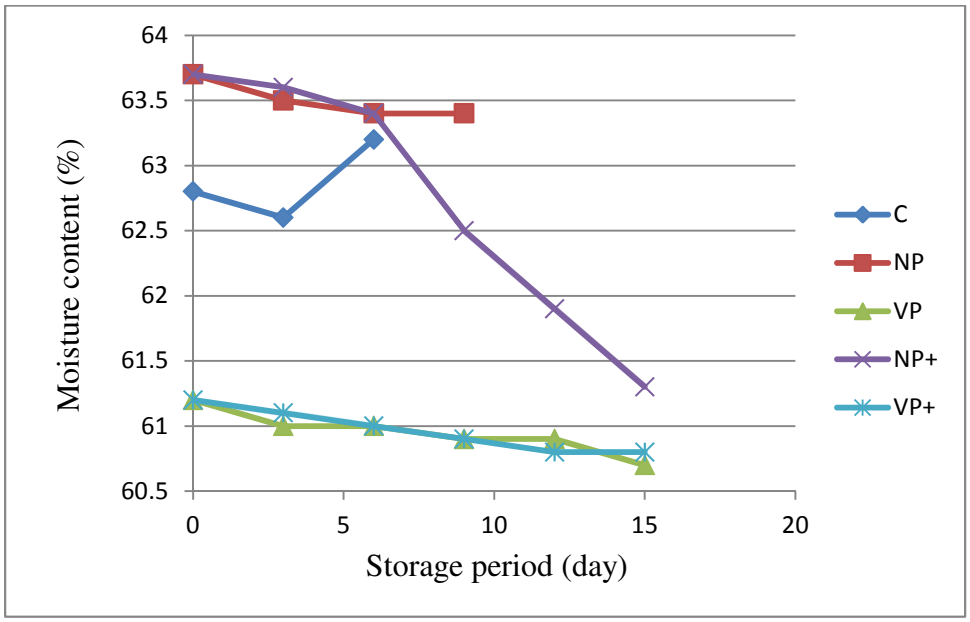
There are also studies which show using of preservatives have an inhibitory effect on yeast and mold growth on the respective samples, as found in this study too. As a results obtained from Rodriguez (2000) shows, addition of preservative had a significant reduction of yeast and mold count on atmospheric air packed bread samples whereas it doesn't bring a change on samples packed under absence of oxygen. This indicates that exclusion of oxygen through packaging as well as using of preservative had closely yeast and mold retardation tendency. This point to using of either of two preservation techniques may have a nearby outcome regarding yeast and mold growth retardation. Even though the results of the current study were not exactly aligned with the above findings (as the target products used are not similar), the path which the results laid are agreeable. Also a result from Ashagrie and Abate (2012) indicates; preservatives applied on injera samples, especially benzoic acid and its sodium salt had a better antifungal activity.

4.3 Moisture content, pH and Color of injera

4.3.1 Moisture content

Results of this investigation showed, there was a significant difference ($p<0.05$) on the moisture content of injera samples with different treatments. As showed in figure 7, the moisture content of injera samples were ranging from 60% -64%.

Figure 7: Moisture content (%) of injera with respective treatments at different storage intervals



For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage respectively since the samples were spoiled.

As indicated on the graph, when the number of days of storage increases, there were gradual decreases in the moisture content of injera samples for all treatments except the control one. The moisture content for the control sample showed a decreasing pattern (alike other treatment samples) till the third day (62.8% to 62.6% on the day of baking and on the third day of analysis respectively). While the result of the sixth day analysis was increased to 63.2%, and this may arise due to the control samples used for the sixth day analysis were somehow moisturized since they were already spoiled by mold growth.

Based on the results, packaging treatment was significantly affected the moisture content of the samples, however addition of preservative was not. As shown on the graph, the moisture content of non-vacuum packed injera samples (NP and NP+) had higher moisture content than vacuum packed and control samples. This may due to the presence of head space left for vapor gas that may in turn condense and results a moisture migration. Whereas the vacuum packed samples (VP and VP+) had lower moisture content, and this may due to the absence of head space. Also, some moisture may sack out together with head space air through evacuation which may result in moisture content reduction.

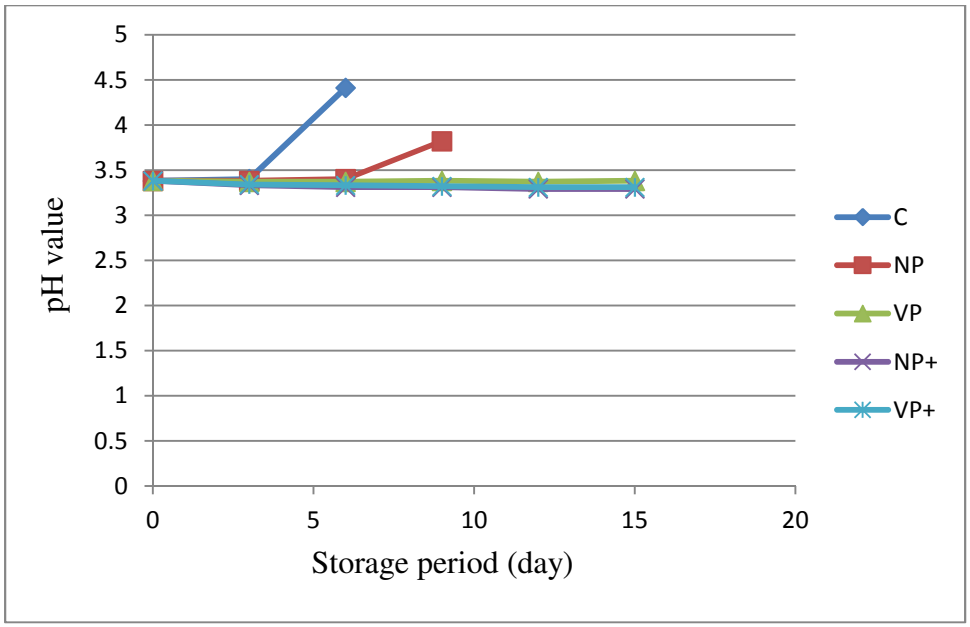
The moisture content of injera samples obtained in this study (60.8 – 63.2%) was comparable with the value reported in the Ethiopian Food composition table (moisture content of injera from different teff verities are 60.2% - 63.8%). Also the result was relative with other findings i.e Ashagrie and Abate (2012), the moisture content of the control injera was 64.8%. The overall mean moisture content of different kinds of bread made from wheat range from 37- 47 % during storage. In the contrary, injera had a higher moisture content that made injera more perishable than most bread (Ashagrie and Abate, 2012).

Moisture is an important parameter in baked foods that significantly affects shelf life and growth of microbial contaminants (Teshome *et al*., 2008). Whereas moisture loss and gain is a serious problem in many bakery products that can result in textural changes and may even promote chemical and microbiological spoilage in low and intermediate moisture products. However, both moisture loss and gain can be overcome by packaging products (Smith *et al*., 2004). In this study also, it was observed that the control sample which is highly exposed for moisture loss and gain were spoiled rapidly than packed samples (non-vacuum packed (NP) samples and Vacuum packed (VP) samples respectively.

4.3.2 pH

Analysis of injera samples under different treatments showed that treatments were not significantly ($p<0.05$) affected the pH value of the samples. As displayed in figure 8, the pH values of the injera samples were found between 3.31and 4.41.

Figure 8: pH value of injera samples through different storage intervals



For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage respectively since the samples were spoiled.

The pH values of injera samples obtained in this study were comparable with the value reported in other related studies. According to Attuquayefio (2014), the result of analysis of the different brands of injera was between 3.65 and 4.02. Also the result reported from Ashagrie and Abate (2012) showed that, the pH of the control sample without preservatives was 3.40 and 3.39 for the sample with sodium benzoate. These results were closer to the results obtained in this study which were 3.38 and 3.34 for control sample and samples with preservative sodium benzoate respectively (at the first date of analysis for each).Whereas the pH values obtained was quite low, when compared with the pH of bread which is mostly between 4.7 and 7.4 (FDA, 2007).

The pH gives an indication of the amount of lactic acid produced during fermentation and hence it determines the sourness of the batter (Attuquayefio, 2014). According to Sahlin (1999), the content of lactic acid at a certain pH is very much dependent on the raw material. Also, environmental conditions such as the favorable pH and moisture content may increase the activity of the flour amylases as well as starch hydrolyzing bacteria. This in turn increases the amounts of fermentable sugars and acid production causing a further decrease in pH. These all

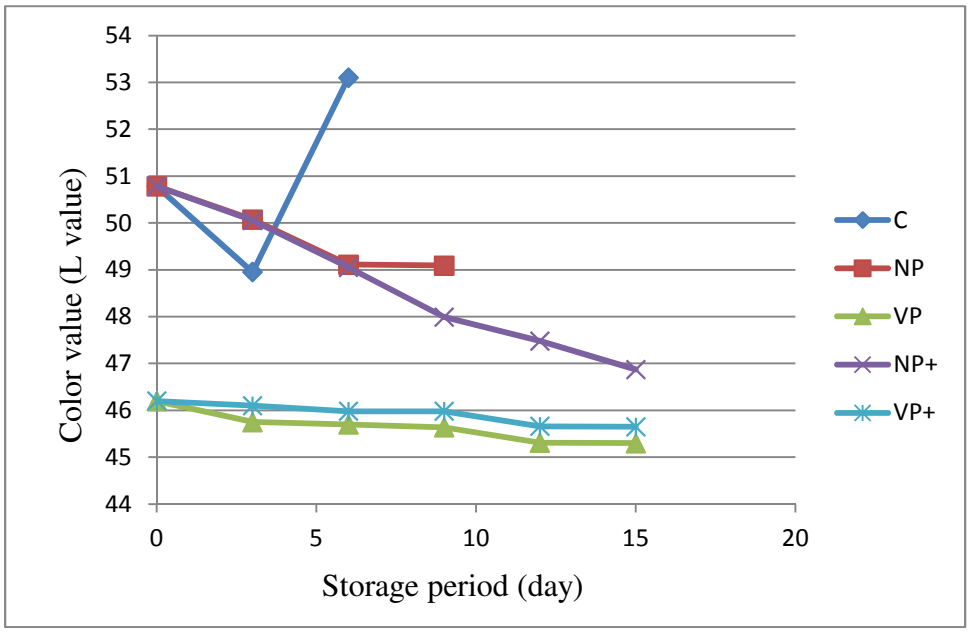
may have contributions to injera be included under high acidic product and the high acidity of injera could account for the few numbers and types of organisms associated with its spoilage.

4.3.3 Color

The superficial appearance and color of food are the first parameters of quality evaluated by consumers, and are thus critical factors for acceptance of the food item by the consumer. Although there are different color spaces, the most used of these in the measuring of color in food is the CIELAB (simply "Lab") color space due to the uniform distribution of colors, and because it is very close to human perception of color (Leon *et al*,2006).

Colour of injera samples as detected by electronic spectrophotometer (Konica Minolita Cm-600d Spectrophotometer) are presented in figure 9. Although the instrument displayed the result with all three CIELAB (L*a*b*) color space values, only L* values were taken to construct this figure since green or blue color spaces were not expected in this product (Injera) while lightness is the more determinant color parameter.

Figure 9: Colour (L value) of injeraat different storage intervals



For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage respectively since the samples were spoiled.

As showed on the graph, when the number of storage day increased, the L* value or lightness of injera samples were decreased gradually for all treatments except the control one. The L value

for the control sample showed a decreasing pattern (alike other treatment samples) till the third day (50.78 to 49.95). While the result of the sixth day analysis was increased to 53.1, and this may arise due to the control samples used for the sixth day analysis were already developed mold, thus whitish mold color may detected.

Spectroscopic color analysis of injera samples under different treatments showed that, packing of the samples with different packaging method were significantly ($p<0.05$) affected the colour (L*) value of the samples whereas application of preservative (sodium benzoate) were not. Based on the results, despite the additive of preservative, vacuum packed samples (VP&VP+) had minimum L value than non-vacuum packed samples (NP&NP+) and control samples (C) respectively. This may be due to evacuation process of vacuum packaging, as the product faced to be distorted and stacked because of the high depressurization which applied for the evacuation process. When the product stacked out or collapsed it may loss its shiny appearance or its lightness. These may the reasons for the colour value of vacuum packed samples become lower and comparatively darker than non-vacuum packed and control samples.

As the result obtained from this study, the L* value of injera samples were ranged from 45 - 53. According to Chere (2018); color of the formulated injera evaluated by the image analysis method, Highest L* value (63.17) was found in formulations with a proportion of 70% teff, 0% maize and 30%rice and least L* value (54.65) was found in 100% pure teff mixture. These results are comparatively higher than the L* value obtained from the present study, which was 50.78 for the control sample at the first day. The reason for lightness difference with the first sample is due to additional rice and maize used (which are obviously lighter than teff). Also in the case of the second sample, the results are closer but not exactly the same. This difference may arise as the methods used for color determination are not the same and variety of teff used has also its own impact. The result reported by Bhol and Bosco (2014) showed that, L* value estimation by color flex for control sample (wheat flour bread) was obtained 75.24, which is agreeable with the result found in this study; since it's obvious that the colour of refined wheat bread is more whitish or lighter than injera (had L* value of 50.78 at the first day of analysis).

4.4 Storage duration of Injera without visible mold growth

The storage duration of injera without visible mold growth was calculated by taking the time from day of baking to the moment in which the samples of a batch presented any visible signs of molding (Table 5).

Table 5: Storage duration of injera without visible mold growth

Treatments	Date of observation for the first visible mold growth	Storage duration
C	6^{th} day	4-5 days
VP	no mold growth until 15^{th} day	more than 15 days
NP	9^{th} day	7-8 days
VP+	no mold growth until 15^{th} day	more than 15 days
NP+	no mold growth until 15^{th} day	more than 15 days

The results obtained from this study indicated that, control samples were started to show visible mold growth on the 4^{th} - 5^{th} day of storage. Whereas the number of visible mold singe -free days was prolonged to more than 15 days for the samples with addition of preservative weather or not packed under vacuum (VP+ and NP+). Also it was extended to 7-8 days for the non-vacuum packed sample with no addition of preservative (NP), and almost doubled when it is packed under vacuum (VP). At the end of the test period (15 days), no molding was observed in samples of three treatments (VP, VP+ and NP+) despite the appearance (figure 10).

Figure 10: Pictures of injera samples (C at 6th day, NP at 9th day, NP+, VP and VP+ at 15th day respectively.

Commonly the expected visible mold sign-free storage period of injera under unpreserved condition is 3-4 days (Ashagrie and Abate, 2012), which is confirmed with this study also. The results of this study revealed, preservative sodium benzoate had a better visible mold sign-free storage period extension effect (>15 dayes) on injera samples weather or not packed under vacuum (NP+ & VP+). Accordingly, these are agreeable and even better than the result reported by Ashagrie and Abate (2012), which indicated that packaging and preservative sodium benzoate gave a better synergetic effect for extending visible mold sign-free storage period of injera.

Also for injera with no added preservative and packed under vacuum (VP), visible mold sign free storage duration was reached to more than 15 days as this method does not favor for mold growth. It has been demonstrated that molds can tolerate O_2 concentration as low as 1% to 2% (Tabak, 1978). Thus, to achieve a significant shelf life extension, the elimination of O_2 from the package should be fast and complete (Piergiovanni and Fava, 1997). Under good vacuum conditions, oxygen in the package headspace is reduced to <1%, i.e., levels which delay mold growth (Smith *et al*., 2004). In this case vacuum packaging becomes a preferable packaging method for the retardation of mold growth and extension of shelf life of products, as it proved with this study too.

4.5 Sensory Evaluation

Taking all sensory attributes tested into account, the results in the current study revealed that there was a statistically significant difference ($p<0.05$) among samples (table 6).

Table 6: Distribution of responses of degree of liking on a 7 point hedonic scale 1-7 (dislike very much- like very much) on sensory attributes of injera samples as regards of treatments

	Mean sensory attributes score (degree of liking)				
Treatment	Color	Texture	Appearance	Taste	Overall acceptability
VP (at day15)	2.00[a]	1.25[a]	1.17[a]	4.83[b]	1.75[a]
VP+ (at day15)	2.17[a]	1.25[a]	1.25[a]	3.67[a]	1.75[a]
NP+ (at day15)	5.83[b]	5.33[b]	5.67[b]	4.33[b]	4.75[b]
NP (at day 7)	5.83[b]	6.08[c]	5.92[bc]	5.92[c]	5.92[c]
C (at day 4)	6.25[b]	6.42[c]	6.33[c]	6.58[d]	6.33[c]

[a-d] *Mean rating values in the same row with different letters are statistically different according to Duncan's test at 5% level of significance.*

As clearly observed n the table; among the treatments, the packaging process had a significant effect ($p<0.05$) on all sensory attributes of the samples. Whereas used preservative sodium benzoate did not bring a significant difference on the sensory attributes of the samples except taste. 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). Despite it happened on other attributes but taste, there was a significance difference between VP and VP+ samples (mean score of 4.83 and 3.67 respectively) as well as NP and NP+ samples (mean score of 5.92 and 4.33 respectively).

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 12 judges was described their degree of sensory acceptance to the injera samples with respective treatments. The colour of injera is one of the most important parameters which mostly catch the first look of the consumers. In areas where injera is consumed as a staple food (Eritrea and Ethiopia), people prefer their injera be white in color. This was also reflected in this study, that the lighter samples were scored higher degree of liking. 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 (Yetneberket al., 2004) in addition to its color

(affects the appearance of the injera in relation to its aesthetic appeal). Texture is also another important parameter which determined by touch and refers to the degree of roughness, smoothness, hardness or softness. According to the result from the current study, vacuum packed sample were scored least degree of liking in terms of appearance and texture that indicates this method affects physical parameters of the product.

The combination of all attributes of the product evaluated by the panelists referred as overall acceptability. In this experiment, results showed that there was a statistically significant difference ($P < 0.05$) in the overall acceptability of injera samples with all treatments. The vacuum packed samples (VP and VP+) had a least mean score on overall acceptability test with a mean score of 1.75, which forced them to be at the very top of Duncan posthok table. Although vacuum packaging is a good technology to extend the mold free shelf life of bakery products, it is not a suitable method for soft bakery products due to its crumpling effect (Smith *et al*., 2004) as happened in this experiment too (Figure 11). Thus, atmospheres with oxygen substituent gas concentrations are ideal, that they do not harm the sensorial characteristics of the product, as it was happened here.

Figure 11: Pictures display a crumpling effect of vacuum packaging, via physical look or appearance of vacuum packed (VP) vs. non vacuum packed (NP) injera samples

Vacuum packed sample (VP) packed look


Non-vacuum packed sample (NP) packed look

Vacuum packed sample (VP) open look

Non-vacuum packed sample (NP) open look

Based on the average mean score of overall acceptability, non-vacuum packed injera samples without and with sodium benzoate (NP and NP+) got the highest mean score (5.92 and 4.75 respectively) following the control sample (6.33). Thus, non-vacuum packed injera samples, especially the one without additional preservative sodium benzoate (NP) got high preference which is compatible with the control sample. This proved that, non-vacuum packaging is a preferable method than vacuum packaging for the objective of retaining sensory quality of the selected product.

5 Conclusions and recommendations

5.1 Conclusions

Injera is the most popular Ethiopian indigenous staple food having a vast growing interest of utilization. Whereas, sustainable supply of the product compatible with the demand becomes very difficult since its shelf life does not usually exceed 3-4 days. As far as we know, preservation of this massive product didn't get a scientific attention it deserves. This study was performed to come up with useful results which are ideal for injera preservation basically from mold spoilage and staling, as they are the major problems affecting shelf life of bakery products including injera.

According to the result of the present study, it was possible to extend the visible mold sign free storage duration of injera to >15 days, using VP+ (vacuum packaging with preservative sodium benzoate), VP (vacuum packaging without preservative sodium benzoate) and NP+ (non-vacuum packaging with preservative sodium benzoate) treatments. Also, the storage duration was extended to 7-8 days with NP (non-vacuum packaging without preservative). Among these, the maximum antimicrobial activity was obtained from VP+, with less arage microbial load which were $5.3*10^1$ bacterial cfu/g and $9.0*10^1$ yeast and mold cfu/g, followed by NP+ ($7.5*10^1$ bacterial cfu/g and $9.0*10^1$ yeast and mold cfu/g). In the present study, staling of injera was determined in terms of moisture content, pH and color. Regarding staling, non-vacuum packaging was found to be effective method than vacuum packaging, as those parameters were highly affected due to its crumbling effect.

The current study also performed the sensory acceptability test of injera at the predetermined visible mold sign free storage period with respective treatments. NP had a better overall acceptability, while VP had a least acceptability (not preferred mainly as sensory attributes were affected due to its crumbling effect). Whereas, sensory preference difference due to chemical additive sodium benzoate was not significant.

Generally, from the results of this study, it was observed that packaging methods and chemical preservative used were significantly ($p<0.05$) affected the shelf life and staling of injera. Apart from being chemical free, vacuum packaging was effective method in mold growth retardation

but it was least effective regarding staling and sensory acceptability. Thus using of non-vacuum packaging method together with chemical preservative sodium benzoate (NP+) was the most effective of all which have better shelf life extension ability as well as sensory quality and acceptability. This implies the possibility of using of chemical preservative sodium benzoate with a reduced amount (exact amount should be studied) for a compatible result obtained by Ashagrie and Abate (2012). This may reduce the problem of frequent consumption related chemical preservative overdoes.

5.2 Recommendations

- ✓ Although vacuum packaging is a good technology to extend the mold free shelf life of injera, it is not a suitable method regarding physical parameters and sensorial characteristics and acceptability due to its crumbling effect. So atmospheres with oxygen substituent gas concentrations are ideal for better result.

    - o A study on possibility of increasing the mold-free shelf life of injera by modified atmosphere packaging (MAP) with various CO_2 and N_2 proportions should be tried in the future.

    - o A study evaluating the shelf life and sensorial character of injera packed with active packaging using O_2 absorbent should be conduct.

- ✓ Further studies should be conduct to assess a suitable injera packaging materials (films) based on their basic features i.e gas permeability and moisture permeability.

- ✓ According to the result of this study, using of non-vacuum packaging method together with chemical preservative sodium benzoate gave a better result than using of preservative alone which was done previously by Ashagrie and Abate (2012). This implies the necessity of further studies on possibility of using of chemical preservatives with a reduced amount for a compatible result.

- ✓ As injera is the most popular Ethiopian indigenous staple food, Ethiopians need it wherever they are around the world. And now a day some foreigners are showing attraction to injera which increase the demand for injera export. Thus, it is better to study the optimum temperature, relative humidity and other environmental factors which lead the way of transportation and storage condition.

- ✓ It is better to use good manufacturing practice (GMP) as well as implementing HACCP (essentially on large scale production areas) in order to reduce and prevent the spoilage of injera.

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Appendix

APPENDIX 1: 7- point hedonic scale sensory evaluation score sheet

You are given servings of injera samples to test and express your degree of liking of each sensory attributes provided. Please test each sample and indicate your response by marking (X) in the parallel space provided. Please rinse your mouth between tastes to remove after taste.

Sample code_____ Date_____

Acceptance with corresponding scale		Sensory attributes				
		Color	Texture	Appearance	Taste	Overall acceptability
Like very much	7					
Like moderately	6					
Like slightly	5					
Neither like Nor dislike	4					
Dislike slightly	3					
Dislike moderately	2					
Dislike very much	1					

Comments_____
_____
_____

Thank you for your participation!

APPENDIX 2: ANOVA for moisture content of injera with respective treatments

```
ONEWAY Moisture BY Treatments
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

Descriptives

Moisture content (%)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
C	3	62.8667	.30551	.17638	62.1078	63.6256	62.60
NP	3	63.4333	.05774	.03333	63.2899	63.5768	63.40
VP	5	60.9000	.12247	.05477	60.7479	61.0521	60.70
NP+	5	62.5400	.97622	.43658	61.3279	63.7521	61.30
VP+	5	60.9200	.13038	.05831	60.7581	61.0819	60.80
Total	21	61.9381	1.13599	.24789	61.4210	62.4552	60.70

Descriptives

Moisture content (%)

	Maximum
C	63.20
NP	63.50
VP	61.00
NP+	63.60
VP+	61.10
Total	63.60

ANOVA

Moisture content (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.676	4	5.419	20.977	.000
Within Groups	4.133	16	.258		
Total	25.810	20			

Post Hoc Tests

Homogeneous Subsets

Moisture content (%)

Duncan

Treatments	N	Subset for alpha = 0.05		
		1	2	3
VP	5	60.9000		
VP+	5	60.9200		
NP+	5		62.5400	
C	3		62.8667	62.8667
NP	3			63.4333
Sig.		.957	.380	.137

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.947.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.