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IMPROVEMENT OF *INJERA* SHELF LIFE THROUGH THE USE OF CHEMICAL PRESERVATIVES

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ABSTRACT

Mould spoilage is a serious problem that affects the shelf life of *injera*, the staple Ethiopian fermented bread. *Injera* is made from teff (*Eragrostis tef*) but other cereals may also be used in combination with teff. About two-third of Ethiopian diet consists of *injera* and it accounts for about two-thirds of the daily protein intake of the Ethiopian population. *Injera* has a high nutritional value, as it is rich in calcium and iron. Unfortunately, *injera* has a shelf life of only 3-4 days essentially due to mould spoilage. The use of weak organic acid as preservative is allowed in acidic foods, primarily as mould inhibitors. In this study, the effect of chemical preservatives such as benzoic acid, sodium benzoate, potassium sorbate and calcium propionate were investigated to prolong shelf life of *injera*. The preservatives were added immediately before baking at the concentration of 0.1% of benzoic acid, 0.1% sodium benzoate, 0.2% of potassium sorbate, 0.3% of calcium propionate and 0.2% blend of the four as recommended by Food and Drug Administration of USA. Three fungal species: *Aspergillus niger*, *Penicillium sp* and *Rhizopus sp* were found to be responsible for *injera* spoilage. *Penicillium* and *Rhizopus* were more dominant at storage temperature of between 16-20^{0}C, while *Aspergillus niger* was found to be more dominant at higher temperature of 25-32^{0}C. *Injera* samples had a pH and moisture content between 3.38-3.45 and 62-65%, respectively. Anti-fungal activities of the preservatives investigated significantly prolonged the shelf life of *injera* for up to12 days. It was found out that the effectiveness of preservation was ranked as sodium benzoate>benzoic acid>potassium sorbate>blend>calcium propionate showing that benzoate and benzoic acid are the most effective. The outcome of the research has a significant implication in food security, energy utilization and a significant reduction in the amount of time used by women to produce *injera*.

Key words: *Injera*, Mould, Spoilage, Chemical, Preservatives

INTRODUCTION

Injera is a thin, fermented Ethiopian traditional bread made from flour, water and starter (*ersho*), which is a fluid saved from previously fermented dough. Teff *(Eragrostis tef (Zucc)* Trotter) is the most popular grain for making *injera*, although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used. Teff [*Eragrostis tef* (Zucc) Trotter] 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 [1]. There is a growing interest in teff grain utilization because of nutritional merits (whole grain); the protein is essentially free of gluten. About two-third of Ethiopian diet consists of *injera* and it accounts for about two-third of the daily protein intake of the Ethiopian population [2].'*Wot*' in the Ethiopian national language (Amharic) means a stew which is made from plant and animal products is served with *injera*. The preparation of teff *injera* consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures. The only required ingredients are the teff flour and water [3]. The method of processing of *injera* from its raw materials to the final product involves preparing and mixing the ingredients to dough, which is fermented and subsequently thinned to a batter. The batter is then poured onto a hot griddle in a thin layer to cook, and to develop colour, flavour and texture [4].The major quality attribute of a good *injera* is its slightly sour taste, which is due to the acidic (low pH)nature of *injera* [5]. Unfortunately, *injera* storage period does not usually exceed three days at ambient temperature (temperature in the highlands of Ethiopia is between 17 and 25^0C) under the traditional storage conditions essentially due to mould spoilage. It is a common practice to discard mouldy *injera*. However, in times of food scarcity, mouldy *injera* is sun dried and prepared for consumption.

Benzoic acid, sorbic acid, propionic acid and their respective sodium, potassium and calcium salts are the most commonly used preservatives in foodstuffs. They are generally used to inhibit yeast and mould growth, being also effective against a wide range of bacteria. These compounds are most active in foods of low pH value and essentially ineffective in foods at neutral pH values [6].

Benzoic acid and its salt have been widely used in the food industry for many years as important food preservatives in order to inhibit various bacteria, yeasts and fungal growth in acidic media. Benzoic acid has been found to cause no deleterious effect when used in small amounts. It is also readily eliminated from the body after conjugation with glycine to form hippuric acid [7].Benzoic acid (C_6H_5COOH) and its sodium salt ($C_7H_5NaO_2$), along with the esters of p-hydroxybenzoic acid (parabens) are permissible in foods at up to 0.1%level [8].

Sorbic acid (C_5H_5COOH) is employed as a food preservative, usually in the form of calcium, sodium, or potassium salt [7, 8]. Sorbic acid and its potassium salt are the most widely used forms of the compounds and are collectively known as sorbates. Sorbic acid and its derivatives are commonly used to inhibit mould growth and extend the shelf life of several foods because of physiological harmlessness and organoleptic neutrality [9].

Propionic acid is employed also as a "rope" inhibitor in bread dough [8]. Propionic acid is a three-carbon organic acid with the structure CH_3CH_2COOH. This acid and its calcium and sodium salts are permitted in breads, cakes, certain cheese, and other foods. Potassium salt is commonly used because it is more stable than the acid form [10]. In general, these three compounds tend to be highly specific against moulds, with the inhibitory action being primarily fungistatic rather than fungicidal.

The inclusion of sorbates or benzoates in the right proportion after completion of fermentation and immediately before baking may reduce the spoilage of *injera* that is due to mould[11,12].There are no reports on the preservative efficiency of the above chemicals in any of the traditional Ethiopian fermented foods. This study, therefore, was designed to evaluate the possibility of using these chemicals at their permissible levels to control mould spoilage and improve shelf life of *injera*.

MATERIALS AND METHODS

Study Site

Experiments were carried out from October 2008 to April 2009 in the Mycological Research Laboratory of the Biology Department and the Food Microbiology Laboratory of Food Science and Nutrition Program, Addis Ababa University where the ambient temperature varied from 10-25^{0}C during the study period.

Preparation of *Injera*

The teff *injera* samples were prepared at home in the same way as done traditionally in every household. Accordingly, teff flour was mixed with clean water in the ratio 1:2 (w/w) and 16 % of starter (*ersho*) by the weight of the flour and was kneaded by hand in a bowl in the traditional way. The resultant dough was allowed to ferment for 3 days at ambient temperature. After this primary fermentation, the surface water formed on the top of the dough was discarded. For every 1kg of original flour, 200ml of the fermented mixture was mixed and with 400 ml of water and brought to boil (traditionally known as '*absit*' making). It was cooled to about 45^{0}C before it was added into the main part of the dough. The main dough was thinned by adding water equal to the original weight of the flour and stirred for 15minutes. The batter was left covered for 2 hours for secondary fermentation. After 2 hours, the *absit* was added to the thinned dough and mixed very well (known as batter making). The batter was left for about 30 min to rise (the second fermentation), before baking commenced. Some more water was added to thin down and form the right batter consistency. Finally, about half a litre of batter was poured onto the hot clay griddle in a circular motion from the outside, working towards the centre. After 2-3 minutes of cooking using traditional baking equipment (*metad*), the *injera* was removed and stored in a traditional basket container *messob*. The *injera* was then transported from home to the laboratory for further study.

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Microbiological Analysis

Isolation and Cultivation of Moulds that Spoil *Injera*

The *injera* samples were kept in the laboratory at ambient temperature for at least 3 days until mould colonies started to appear visually on its surface. After three days of storage at ambient temperature of 10-25^{O}C, depending upon their difference in color and other colony morphology, the spoilage moulds were directly transferred into potato dextrose agar (PDA) that contained 60mg/l Chloramphenicol as an antibacterial agent. The cultures were then incubated at room temperature to induce the growth of the fungi for five days. To get a pure culture, each of the emerging colonies was transferred aseptically to fresh PDA agar plates for identification. The fungi were maintained on PDA agar slants in the refrigerator (4^{0}C).

Characterization and Identification of Moulds that Spoil *Injera*

Identification to the genus level of the fungal isolates was based on morphological characterization that places emphasis on colony characteristics, spore size and shape and vegetative morphology of isolates within genus level [13]. For this purpose, slide cultures were prepared for each of the isolates.

Effect of Temperature

Temperature optima for the common *injera* moulds were assessed by inoculating the moulds on *Injera* Sucrose Agar (ISA) media. ISA media were prepared in the following way: freshly baked *injera* was sun-dried and changed to '*derkosh*' (dried *injera*). The '*derkosh*' was powdered using mortar and pestle; 100g of the powder was mixed with 500ml of water and kept in a shaker for 15 min. The *injera* broth was filtered using cheese cotton cloth, followed by addition of broth (2%) sucrose and 2% agar; the mixture was then heated until it boiled. After sterilization (15 min at 121^{0}C), the media was poured on Petri dishes. ISA media were kept in a refrigerator for future practical studies on *injera* moulds. The growth and sporulation of *Aspergillus niger*, *Penicillium* and *Rhizopus* were assessed by inoculating spores on ISA media. The cultures were incubated at temperatures of 4^{0}C (refrigeration temp), 16^{0}C (average room temperature during the experiment days), 20^{0}C, 25^{0}C and 32^{0}C. The growth (colony size) of each of the moulds at different temperatures was determined and recorded as no growth (-), slight mould growth (+), moderate mould growth (++) and high mould growth (+++) after 5 days of incubation. A triplicate of each of the fungi on agar plates was used for each temperature treatment.

Determination of pH and Moisture Content of *Injera*

An electronic pH meter (Jenway Model 370 pH/mV Meter, England) was used to measure the pH of *injera*. The moisture content of the samples was determined by drying 10g of sample in an air oven at 105^{o} C to constant weight (16-18 h) [14].

Evaluation of Efficacy of Chemical Preservatives

Addition of Chemical Preservatives

Each of the chemical preservatives were added on the *injera* batters at the concentration of 0.3% calcium propionate, 0.2% potassium sorbate, 0.1% benzoic

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acid, 0.1% sodium benzoate and 0.2% blend of the four preservatives just immediately before baking. These values correspond to 1.5 grams of propionate, 0.5 grams of benzoate and 1 gram of sorbate per 500 g of flour [8]. After the baking process, the experimental and control *injera* samples were stored in the laboratory at ambient temperature.

Comparing the Rate of Mould Invasion

Mould invasion on each of the treated *injera* samples and the control was monitored daily over a period of 12 days. *Injera* rolls were left at room temperature and a photo of each was taken. *Injera* spoilage was evaluated based on the percentage of the total surface area of each roll where fungal invasion occurred. The measurement of the mould outgrowth from the photos was done using the Photoshop elements of *Adobe Photoshop Version 10.0*. An *injera* roll was deemed positive if more than 1% of the total surface area was covered with fungi [15].

Evaluation of Shelf Life of *Injera*

The *injera* samples were examined for visible signs of mould growth on the crust every day. The microbial shelf life is defined as the period in days during which the spoilage caused by microorganisms was first observed. The shelf life was expressed in relation to the corresponding control [16].

RESULTS

Moulds Responsible in *Injera* Spoilage

In this study, small white fungal colonies (visible to the naked eye) appeared on *injera* starting on the 4^{th} day of storage in a *messob*. Accordingly, the small colonies gradually grew and started to show different kinds of colony color on sporulation. The *injera* moulds were isolated at different times of storage at ambient temperature and viewed using a microscope after observing morphological characteristics of the colony. It was then found that three types of fungal species belonging to the genera of *Penicillium, Aspergillus (Aspergillus niger)* and *Rhizopus* species were responsible for *injera* spoilage, although their extent of growth varied depending on temperature.

Effect of Temperature on the Growth Rate of *Injera* Moulds

This study revealed that *Penicillium* and *Rhizopus* were more dominant in spoiling *injera* at lower temperature, while *Aspergillus niger* grew much faster as the temperature gets higher (25-32^{0}C). None of the moulds grew when the temperature was kept at 4^{0}C (Table 1).

pH and Moisture Content of *Injera*

pH of *Injera*

Results of this investigation showed that *injera* was generally acidic irrespective of the type of preservatives added (Table 2). The acidity of samples stored at ambient temperature ranged from pH 3.45 to 3.38. The pH values were 3.38 for the sample with benzoic acid, 3.39 for the sample with sodium benzoate,3.45 for the sample with potassium sorbate and 3.44 for the sample with calcium propionate. The pH of the control sample without preservatives and blend of all the four preservatives sample were 3.40 and 3.38, respectively.

Moisture Content of *Injera*

The moisture content of fresh *injera* samples (as soon as it was baked) varied from 62-65% (Table 3). There was no significant difference (p >0.05) in the moisture content of *injera* with and without preservatives. However, there was a gradual decrease of around 1 % in the moisture content with duration of storage from the 1stto the 5th day at ambient temperature in traditional storage.

Efficacy of the Chemical Preservatives on *Injera*

Extent of Mould Invasion on *Injera*

In this study, it is shown that the chemical preservatives were effective in inhibiting moulds responsible for *injera* spoilage. This was shown by the reduction in percentage of mould invasion of the samples containing preservatives as compared to the sample without preservative, the control (Figure 1). The effectiveness of the organic acids used in this study generally increase in effectiveness in the order of propionic acid <sorbic acid < benzoic acid.

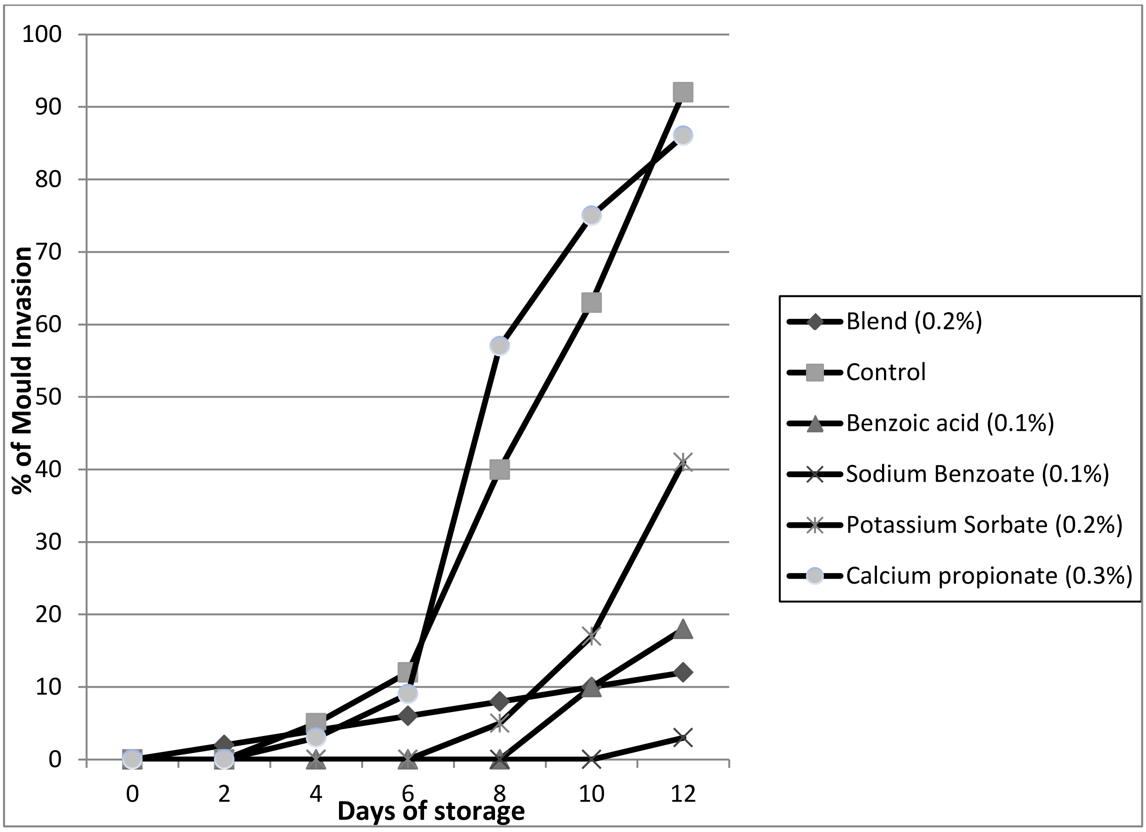

Figure 1: Mould invasion of *injera* samples in 12 days of storage

Shelf Life of *Injera* with and without Preservatives

The effect of chemical preservatives on the shelf life of *injera* is shown in Table 4 and it was determined as the day in which mould was visible to the naked eye. The shelf life of *injera* without preservatives (control) was 3-4 days while *injera* containing 0.3 % calcium propionate was 4-5 days depending on the storage temperature. Shelf life of *injera* containing 0.1 % benzoic acid and sodium benzoate were the better preservatives of all tested chemical agents, which prolonged the shelf life of *injera* up to 12 days. Potassium sorbate and blend of the four preservatives at the concentration of 0.2 % similarly prolonged the shelf life of *injera* from 6-8 days at 20 ± 2^0C.

DISCUSSION

A variety of fungal species are responsible for the spoilage of each kind of food [17]. In bakery products, the baking temperature is generally sufficient to destroy these organisms; contamination might arise from mould spores derived from the atmosphere or from surfaces during the cooling, finishing and wrapping procedures [18, 19]. In case of freshly made *injera*, it is free of fungi but the large surface area of the flat bread provides high probability of fungal contamination from the air.

The reduced activity of a fungus at lower temperature may have corresponded with reduction of nutrient utilization, so that the fungus used the colonized substrate more slowly. *Aspergillus niger's* faster growth on ISA media as the storage temprature raised from 25^0c to 32^0c(Table 1) is consistent with previous reports. *Aspergillus niger* require a higher temperature with an optimum temperature range of 28-34°C for its growth [19], which is why *Aspergillus niger* grows better in warmer climates[20].From the literature, it is apparent that most fungi are mesophilic and are inhibited or killed at unusually high or low temperatures. There are some, however; that actually require unusual temperatures: thermophiles (>40^0C) and psychrophilic (15^0C or less**)** [21].Obviously from the analysis of the study the moulds spoiling *injera* were mesophiles since their growth temperature ranged between 16 and 32^0C. One of the possible explanations for the isolated moulds to be only mesophiles could be related to the climatic condition of the study site, where a moderate climatic condition of maximum temperature of 24.6^0Cwas recorded. Contamination of moulds is less important in cool highland regions than warm lowland regions [5].With a significant altitude difference in Ethiopia, obviously the dominant mould spoiling *injera* might vary from the colder to the hotter parts of the country.

Even though bakery products are important staple foods in most countries and cultures, mould growth and drying are two problems that limit the shelf life of high and intermediate- moisture baked foods [22]. In this study, the moisture content of *injera* had decreased slightly when kept for 5 days. Even though this study concentrated on *injera* baked at home, the moisture content of the control *injera* was comparable with the value reported in the Ethiopian Food composition table of Ethiopian Health and Research Institute [23]. From the composition in the table, it can be seen that *injera* made from any of the cereals has higher moisture content than any of the bread types.

The overall mean moisture content of different kinds of bread made from wheat range from 37-47% during storage [24]. On the contrary, *injera* had a higher moisture content of 62-65% (Table 3), a factor that is responsible for higher susceptibility of *injera* to mould growth. Moreover, *injera* provides larger surface area to weight ratio for fungal spores to land and grow. To prevent mould growth, the product should be properly cooled and dried before packing [5].However, these practices are not applicable to *injera* preservation.

Anti-fungal activity of preservatives investigated in this studyshowed that *injera* treated with benzoic acid and its sodium salt were best preserved compared to others with different treatments. Even after 12 days of storage, samples containing benzoic acid and its sodium salt had better keeping quality (Figure 1) than samples with other preservatives. Samples without preservatives (control), however, exhibited the highest mould invasion visible from 4-12 days of preservation and consequently, had a shorter shelf life compared to those with preservatives(Table 4).Moulds are relatively tolerant to low pH, low water activity, low temperature and the presence of preservatives[25].

The effectiveness of the organic acids used in this study against mould growth generally increased in the order of propionic acid <sorbic acid < benzoic acid which is in agreement with the results of Gould (1996) who indicated that the order reflects their increasing lipophilicity [26]. Benzoic acid and its salt (sodium benzoate) were the most effective preservatives in delaying the spoilage of *injera* as compared to the other preservatives tested in this study. This could be due to the fact that anti-microbial activity of benzoic acid is essentially in the undissociated form (pKa =4.2) which has the ability to interface with membrane energetic[27]. Moreover, benzoic acid is relatively acidic, which supports the inhibition of the growth of spoilage moulds. Further, it can be speculated that the higher effectiveness of sodium benzoate as compared to benzoic acid could be due to the fact that the salt is 200 times more soluble than the acid.

The lower effectiveness of propionic acid in contrast to benzoic acid and sorbic acid might be due to the resistance of some species of moulds and their growth in media containing substantial amounts of propionic acid and its salts. Moreover, when compared to propionic acid and sodium propionate, calcium propionate was least effective as an inhibitor of mould growth [28].

The optimum pH level for the inhibitory effect of propionic acid is 5.0 with a maximum effect at pH of 6.0 [16]. However the pH of *injera* containing calcium propionate is highly acidic (pH=3.44) (Table 2), which could be one reason for its low effectiveness. The second reason for the low effectiveness of calcium propionate on *injera* could be the concentration used. Propionic acid should only be added to bread in a concentration not exceeding 3000ppm and this concentration was used in this study. However, recent studies have shown that under these conditions, propionic acid is not effective against common bread spoilage organisms[15].

The combined (blended) effect of the four preservatives used for this study was also evaluated. The combined action of two or more preservatives is more effective in inhibiting the growth of microorganisms, which could be due to a synergistic effect among the preservatives [29]. However, in this study there seems to be no synergistic effect since the blended sample was only more effective than calcium propionate and less effective than benzoic acid, sodium benzoate and potassium sorbate.

CONCLUSION

Fungi are the most common spoilers in bakery products. Commonly, a shelf life of 3-4 days may be expected when they are unpreserved. The preservatives used in this study had prolonged the shelf life of *injera* for up to 12 days. It was evident that the visible mould growth became clear only after the 10th day. In these 10 days, the *injera* could be consumed before it loses its acceptability. However, further study on the sensory quality of injera after these 10 days should be investigated. The outcome of this research has a significant implication for food security, energy utilization and a significant reduction in the amount of time required by women to produce *injera*.

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Table 1: Growth Rate of *Injera* Moulds at Different Temperature on Media Made From *Injera*

	Growth of moulds under different temperature (^{0}C)				
Moulds	4 ^{0}C	16 ^{0}C	20 ^{0}C	25 ^{0}C	32 ^{0}C
A.niger	-	-	+	++	+++
Penicillium spp	-	+++	++	+	-
Rhizopus spp	-	+++	++	+	-

– No growth + Slight mould growth
++ Moderate mould growth +++High mould growth

Table 2: pH of *injera* with and without preservatives

***Injera* samples**	**pH**
Control	3.40
+ 0.1 % benzoic acid	3.38
+ 0.1 % sodium benzoate	3.39
+ 0.2 % Potassium sorbate	3.45
+ 0.3 % calcium propionate	3.44
+ 0.2 % Blend of the four	3.38

Table 3: Moisture Content of the *Injera* with and without preservatives

Injera samples	Moisture content	
	1st day	5th day
Control	64.2 %	63.6 %
+ 0.1 % benzoic acid	63.7 %	62.3 %
+ 0.1 % sodium benzoate	63.4 %	62.5 %
+ 0.2 % Potassium sorbate	64.3 %	61.8 %
+ 0.3 % calcium propionate	64.4 %	63. 1 %
+ 0.2 % Blend of the four	63.5 %	61.2 %

Table 4: Shelf life of *injera* containing preservatives at 20$\pm$ 2^{0}c

Injera Treatments	Shelf life (days)
Control	3-4 days
+ 0.1 % benzoic acid	8-10 days
+ 0.1 % sodium benzoate	10-12 days
+ 0.2 % potassium sorbate	6-8days
+ 0.3 % calcium propionate	4-5 days
+ 0.2 % blend of the four	6-8 days

REFERENCES

1. **Central Statstical Agency of Ethiopia**. Agricultural Sample Survey: Report on Area and Production of Crops.Addis Ababa. 2008.

2. **Arogundade LA** Functional characterization of teff (*Eragostics tef*) protein concentrate: Influence of altered chemical environment on its gelation, foaming, and water hydration properties. *Food Hydrocolloid.* 2006;**20**:831-838.

3. **Gashe BA** Involvement of lactic acid bacteria in the fermentation of teff (*Eragrostis tef*), an Ethiopian fermented food. *J. Food Sci.* 1985;**50**:800-801.

4. **Fellow P** *Traditional Foods: Processing for profit.* Intermediate Technology Publications. London.1997.

5. **Zegeye A** Acceptability of *injera* with stewed chicken. *Food qual.prefer.*1997**;8 (4):** 293-295.

6. **Santini AO, Pezza HR, Filho OC, Sequinel R and L Pezza** Potentiomertic sensor for sorbic acid determination in food products. *Food Chem.* 2009. doi: 10.1016/j.foodchem.2009.02.012.

7. **Doughari JH, Alabi G and AM Elmahmood** Effect of some chemical preservatives on the shelf life of sobo drink. *Afr. J. Microbiol. Res.*2007; **2**:37-41.

8. **Jay JM** Modern Food Microbiology: Aspen Publishers. Maryland 2000.

9. **Venturini MM, Blanco D and R Oria** In vitroantifungal activity of several antimicriobial compounds against *penicillium expansum. J.Food Protect.* 2002; **65(5):**834-839.

10. **Gonzalez-Fandos E and JL Dominguez** Effect of potassium sorbate washing on the growth of *Listeria monocytogenes* on fresh poultry. *Food Control*, 2007;**18**:842-846.

11. **Ashenafi M** The microbiology of Ethiopian foods and beverages:a review. *SINET: Ethiop.J. Sci.* 2002;**25**(**1**):97-140.

12. **Ashenafi M** A review on the microbiology of indigenous fermented foods and beverages of Ethiopia. *Ethiop.J Biol. Sci*. 2006;**5(2):**189-245.

13. **Barnett HL and BB Hunter** Illustrated Genera of Imperfect Fungi. Burgess publishing company Minneapolis. 1972.

14. **AOAC.** Association of Offical and Analytical Chemists. Official Methods of Analysis (17th ed., Vol. 2). Washington D.C. 2000.

15. **Ryan LM, Dal Bello F and EK Arendt** The use of sourdough fermented by antifungal LAB to reduce the amount of calcium propionate in bread. *Int. J. Food Microbiol.* 2008. **125**:274-278.

16. **Katsinis G, Rigas F and D Doulia** Synergistic effect of chemical preservatives with ethanol on the micriobial shelf life of bread by factorial design. *Int. J. Food Sci.Techn.* 2008;**43**:208-215.

17. **Filtenborg O, Frisvad JC and U Thrane** Moulds in food spoilage. *Int. J. Food Microbio.* 1996; **33(1):**85-102.

18. **Gock MA, Hocking AD, Jonh I, Pitt JI and PG Poulos** Influence of temperature, water activity and pH on growth of some xerophilic fungi. *Int. J. Food Microbiol*. 2003; **2481**:11-19.

19. **Tyson JL and RA Fullerton** Effect of soil-borne inoculum on incidence of onion black mould (*Aspergillus niger*). *New Zealand Plant Protect.*2004;**57**:138-141.

20. **Shur KI and PV Nielsen** Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. *Int. J. Food Microbiol.*2004;**95**:67-78.

21. **Malloch D** Moulds:Isolation, Cultivation and Identification.University of Toronto, Toronto. 1981.

22. **Guynot ME, Marin S, Sanchis V and J Ramos** Modified atmoshere pacaking for prevention of mold spoilage of bakery products with differnt pH and water activity. *J.Food Protect.*2003; **66(10):**1864-1872.

23. **EHNRI.** Ethiopian Health and Nutrition Research Institute.Food compostion table for use in Ethiopia. Addis Ababa (1997).

24. **Ayub M, Wahab S and Y Durrani** Effect of water activity (aw),moisture content and total microbial count on the overall quality of bread. *Int. J. Agric.Bio* .2003; 274-278.

25. **Huis in't Veld J** Microbial and biological spoilage of foods: an overview. *Int. J. Food Microbiol*. 1996;**33**:1-18.

26. **Gould GW** Methods of preservation and extension of shelf life. *Int. J. Food Microbiol*. 1996;**33**:51-64.

27. **Akpan UG and AS Kovo** Preservation of passion fruit juice. *Leonardo J.Sci.* 2005; **7**: 17-22.

28. **Razavi-Rohani SM and MW Griffiths** Antifungal effects of sorbic acid and propionic acid at differnt pH and NaCl conditions. *J. Food Safety*. 1999; **19**:109-120.

29. **Martrtinez-Flores HE, Gaytan-Martinez M, Figueroa-Cardenas JD, Martinez-Bustos F, Reyes-Vega ML and A Rodriguez-Vidal** Effect of some preservatives on the shelf life of corn tortillas obtained from extruded masa. *Agrociencia*. 2004;**38(3):**285-292.