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Research Article

Improvement of Injera Shelf Life and Staling through Vacuum and Nonvacuum Polyethylene Packaging: Their Synergistic Effect with Chemical Preservative

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The application of vacuum packaging (VP) and nonvacuum 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+ (nonvacuum packaging with preservative) treatments. Among these, VP+ had the 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 the least average scoring of MC, pH, and *L* value (60.96%, 3.33, and 45.92, respectively) and sensory acceptability, basically due to the crumbling effect of the packaging method used. Besides, NP + had a 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 a relatively effective method regarding sensory acceptability and staling as it had 62.73%, 3.32, and 48.70 average MC, pH, and *L* value, respectively. Generally, packaging methods and preservative used were 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.

1. Introduction

In most nations and civilizations, bakery goods are significant staple foods [1]. They are baked foods made of flour, such as pies, cakes, pastries, and bread. Due to physical, chemical, and microbiological causes, bakery products can degrade. Mold growth and its complications cause a significant microbiological loss in bakery products [2]. The primary bakery goods are bread and cake. In general, commercially prepared and properly stored bread does not contain enough moisture to support the growth of any creatures but mould. Rhizopus stolonifer, commonly referred to as the "bread mold," is one of the most prevalent molds that develop and contaminate bread. Low humidity conditions slow down bread storage [3].

East African injera, a sourdough-risen flatbread typically produced from the small, round, iron-rich grain known as teff, served as the nation's dish in Ethiopia and Eritrea. However, because to the infrequent production and high cost of teff, wheat, barley, corn, and/or rice flour is

occasionally used to replace all of the teff content [4]. The primary ingredients are flour, water, and sourdough starter (ersho), which is a liquid saved from the previously fermented dough. Then, an ingredient mixture was allowed to ferment before being baked on a ceramic plate. Then, it is served with a variety of stews (wot) or sometimes salads (Cooking of Science, 2008).

Since injera is acidic, its key quality characteristic is a somewhat sour flavor [5]. However, injera's acceptance and palatability are also influenced by the desirable texture. Unfortunately, the injera's shelf life and its superior qualities only last 3 to 4 days [6]. This could result from the fact that a product's spoilage and quality loss elements can interact directly under standard storage conditions. These necessitate the modification of methods that can lessen those elements' direct interaction with the product; packing is one such vital method [7].

Keeping food at the desired level of qualities or nature for their maximum advantages is known as food preservation [8]. The use of various preservation methods by itself helps to preserve food. Whereas it is preferable to preserve food using a combination of several preservative techniques (such as heating, cooling, drying, salting, curing, acidification, oxygen removal, and fermentation) in order to maintain its sensory and nutritional quality as well as its microbial stability and safety [9].

Packaging is one of the preservation methods which increase the shelf life of manufactured foods and ensuring food safety [10]. Among these technologies, vacuum packaging (VP) and modified atmosphere packaging (MAP) are used to prevent products from contamination and evaporative losses and also extend the storage life of food products [11].

One of the best techniques to lengthen the shelf life of food goods is vacuum packaging. The preservative effect of VP is due to the environment being made oxygen-deficient, which severely or completely inhibits potential spoilage organisms. By removing air from around the product, the levels of oxygen in the packaging are reduced to a nonsignificant amount [3]. The lack of oxygen then delays the ability of oxygen-breathing microorganisms to grow and spoil the product. Moreover, vacuum packaging reduces the amount of spoilage due to oxidation [12]. Vacuum packaging protects 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 [13].

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The preservative effect of VP is due to the environment being made oxygen-deficient, which severely or completely inhibits potential spoilage organisms.

The packaging's oxygen levels are decreased to negligible levels by eliminating the air surrounding the goods. Because of this, oxygen-breathing microorganisms have less time to develop and ruin the product. In addition, vacuum packaging lessens oxidation-related deterioration. The food inside vacuum packing is protected from external factors including moisture and oxidation processes, which allows the food to stay fresh and of high quality for a much longer period of time. In addition, vacuum packaging can reduce the packaging's volume [13].

1.1. Statement of the Problem. The primary and most popular cuisine in Ethiopia is injera, which makes up around two-thirds of the country's diet [14]. In addition, consumption of injera has been rising over time due to growing interest in and knowledge of its nutritional virtues, including its gluten-free protein, iron, and calcium content, as well as its flexible taste and other advantages.

Injera typically has a shelf life of no more than three days at room temperature. In order to address this issue, Ashagrie and Abate [6] did a study on the enhancement of injera shelf life with chemical preservatives. This study found that injera with chemical preservatives had a shelf life of 4–12 days compared to 3-4 days for injera without preservatives (control). In addition, the percentage of mold infestation was lower in the samples with preservatives compared to the samples without preservatives, demonstrating the efficiency of chemical preservatives in preventing the molds that cause injera to decay.

Even though the aforementioned study achieved positive results in terms of extending the shelf life of injera, concerns about toxicity and the demand for organic (additive-free) products remain. This is because injera is a staple food that is frequently consumed, which forces the dose of chemical preservatives added to increase proportionately and cause toxicity. Another issue was the product's dryness. These may make it necessary to replace it with other preservation procedures or combine it with other strategies for enhanced synergy.

The majority of the literature's findings largely concur that aerobic mold growth is the primary factor contributing to the deterioration of bakery goods [15–17]. The same applies to Injera [6]. This can be as a result of the conventional storage conditions, which are appropriate for oxygen dominance in the storage environment. Therefore, because they are utilized to keep oxygen and moisture out, packing techniques including vacuum and nonvacuum polyethylene packaging may be used as effective preservation approaches [18]. Additional factors for choosing this preservation method (packing) include the inapplicability of standard preservation methods for bakery items, such as pasteurization or sterilization [19] and the need of technology which aids the portability of product for export and distribution. Therefore, the objective of this study is to improve injera shelf life and staling using vacuum and nonvacuum polyethylene packaging together with a chemical preservative.

2. Materials and Methods

2.1. 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 [20]. Teff grain (called *nechi* teff) were

purchased from the 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 a ratio of 1 : 3 (*v*/*v*) and cooked for 15 minutes with continuous stirring. The cooked batter were left to cool to 45°C 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 a smooth and clean table covered with a 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., nonvacuum packed (NP), Vacuum Packed (VP), nonvacuum packed with preservative (NP+), Vacuum packed with preservative (VP+), and control I) were packed accordingly and sent to AASTU Department of FSAN laboratory and stored there at room temperature for further analysis.

2.2. 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 [6].

2.3. Pre-Packaging Activities. Well cooled injera for the 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 the 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.

2.4. Method for Vacuum and Nonvacuum 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 [21]. The instrument is used to create a vacuum and simultaneously seal the pack in a 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 nonvacuum 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.

2.5. 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.

2.6. Microbial Analysis

2.6.1. 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 a 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 the preparation of PCA plates, 1 ml of diluted sample was dropped on the center of the 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°C for 2 days and at 25°C for 5 days, respectively.

2.7. Bacterial Count. The aerobic Plate Count (APC) method was performed as an indicator of the 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 the 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 [22].

2.8. Yeast and Mold Count. Diluted sample (0.1 ml) were inoculated onto potato dextrose agar (PDA) medium supplemented with 60 mg/l chloramphenicol (in order to suppress the growth of bacteria) using a 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 [22]. 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.

2.9. Physico-Chemical Analysis

2.9.1. Moisture Content Determination. The moisture content (MC) of the samples was determined by oven drying method. 5 g of portion of the sample (well homogenized sample taken from all the quarters) were dried in a hot air oven at 105°C for three hours and the drying was proceeding until constant weight was obtained [23].

2.9.2. 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 10 g of ground injera with 100 ml distilled water) was measured [11].

2.9.3. Color Determination. The samples for color determination were taken to the AAU department of chemical and Biological engineering instrumental laboratory and the color were determined by an 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 [24].

2.10. 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 [25].

2.11. Sensory Evaluation. The samples for sensory analysis were prepared and packed as the same way has carried out 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 the 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 semitrained panelists (Heymann et al., 2012); from students and staffs of AASTU, who has knowledge about the 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.

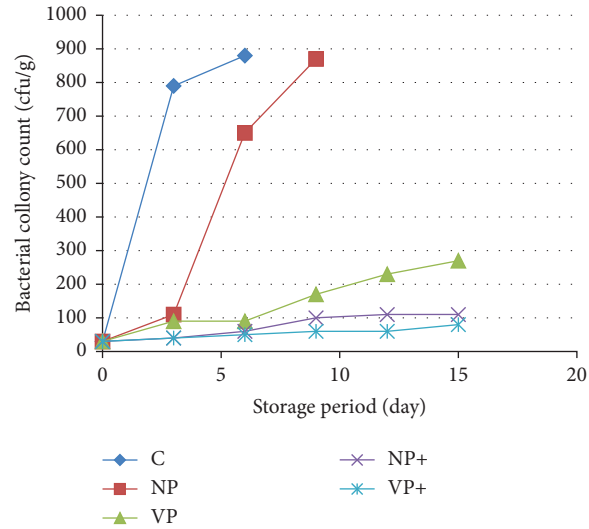

Figure 1: Colony forming units of bacteria/g of injera with respective treatments at different storage intervals. For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage, respectively, since the samples were spoiled.

2.12. 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 posthoc test was applied to rank the mean values of different treatments as computed by SPSS (version 20.00) software.

3. Results and Discussion

3.1. 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 Figure 1. The graph shows the patterns of bacterial exposure of injera, as determined by the agar plate technique.

Treatments had significantly ($P < 0.05$) affected the bacterial count of injera. As shown 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 nonvacuum packed injera without preservative (NP)). A 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 postbaking contamination. Since the unpacked injera doesn't have a protection from exposure to the external environment, it had a higher possibility for environmental contamination. In addition, the exposure to environmental oxygen was also another factor which aids the bacterial growth.

Likewise, nonvacuum packed injera without preservative (NP) shows a 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 postbaking contamination. However, as the packaging method used relatively allows head space oxygen, it played a positive role to support bacterial growth on injera samples packed under this treatment (NP).

In the case of nonvacuum 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 the most effective treatment against bacterial spoilage followed by NP+. This indicated that applying a chemical preservative (sodium benzoate) as well as using the vacuum packaging method gave a better control against bacterial inhibition.

The addition of the chemical preservative sodium benzoate also had an impact on a bacterial colony count of prebaked batter. The bacterial colony counts of injera 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 an 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 the 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 [15, 16, 19, 26, 27]. For example, as Sourki et al. [16]; bread slices packed under CO_2/N_2 shows significant bacteriostatic and fungi static properties. This shows exclusion of oxygen in one or another 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

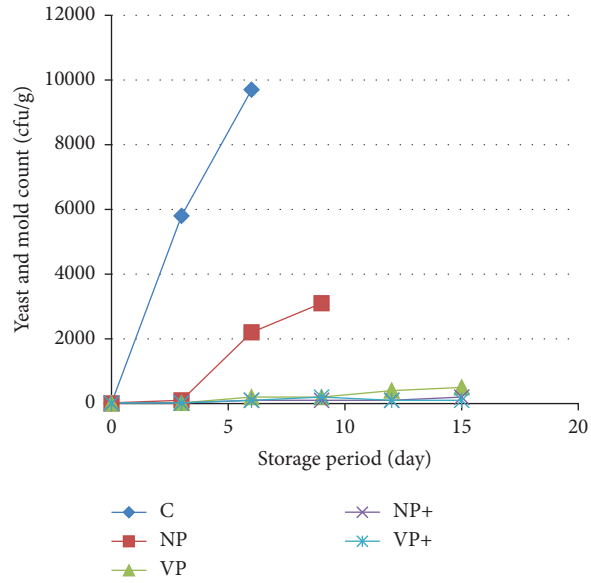

Figure 2: Colony forming units of yeast and mold/g of injera with respective treatments at different storage intervals. For "C" and "NP" treatments, the experiment was terminated at 6^{th} and 9^{th} day of storage, respectively, since the samples were spoiled.

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 the numbers of bacteria in the product [28].

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 the most active against yeasts and bacteria [29].

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. [30]; Average counts of total heterophilic 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 a pH less than 4.5 (acidic range which is not suitable for bacterial growth) whereas bread has a 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 [31]. Total heterotrophic counts indicate the 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 [32].

3.2. 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 2. The graph shows the patterns of yeast and mold count of injera across the storage period.

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 nonvacuum packed injera without preservative (NP)). Relatively, maximum numbers of yeast and mold colonies was observed in the control sample followed by the 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 the 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 the highest number following the control sample. This higher increment of yeast and mold colony count were arised from the effect of postbaking contamination and the 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 that 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. Besides these, applying a chemical preservative (sodium benzoate) gave a superior synergetic effect on yeast and mold growth retardation. Thus, Vacuum Packed injera with preservative sodium benzoate (VP+) as well as nonvacuum packed injera with preservative sodium benzoate (NP+) were proved to be the most effective treatments against yeast and mold growth.

The addition of the chemical preservative sodium benzoate also had an impact on the yeast and mold colony count of prebaked 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 an increase in time of exposure. Whereas, the yeast and mold colony count were vastly reduced due to baking. At the day of baking, the 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 the O_2 amount has a significant mold growth inhibitory effect. According to Khanom et al. [30]; the total yeast and mold

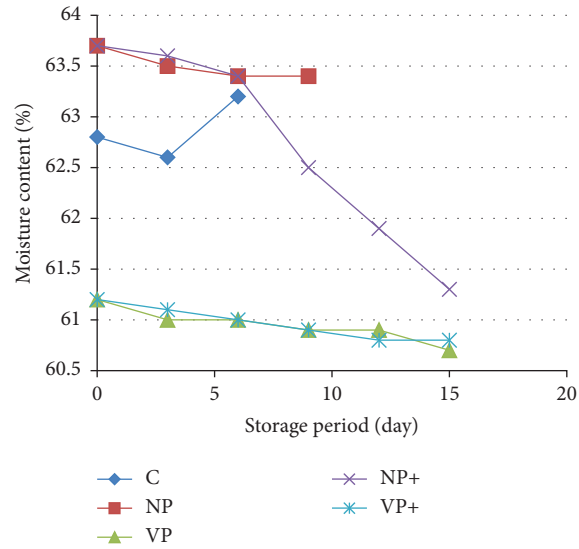

Figure 3: Moisture content (%) of injera with respective treatments at different storage intervals. For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage, respectively, since the samples were spoiled.

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 et al. [15]; 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 the absence of oxygen (replaced by a 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, nonvacuum 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% [33].

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 the results obtained from Rodriguez et al. [15] shows, the 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 the absence of oxygen. This indicates that the 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-given findings (as the target products used are not similar), the path which the results laid are agreeable. Also, a result from Ashagrie and Abate [6] indicates; preservatives applied on injera samples, especially benzoic acid, and its sodium salt had a better antifungal activity.

4. Moisture Content, pH, and Color of Injera

4.1. Moisture Content. The results of this investigation showed, there was a significant difference ($P < 0.05$) on the moisture content of injera samples with different treatments. As shown in Figure 3, the moisture content of injera samples were ranging from 60%–64%.

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 nonvacuum 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 [6]; 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 to 47% during storage. In the contrary, injera had a higher moisture content that made injera more perishable than most bread [6].

Moisture is an important parameter in baked foods that significantly affects the shelf life and growth of microbial contaminants [34]. 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 [13]. In this study also, it was observed that the control sample which is highly exposed for moisture loss and gain were spoiled more rapidly than packed samples (nonvacuum packed (NP) samples and Vacuum packed (VP) samples, respectively).

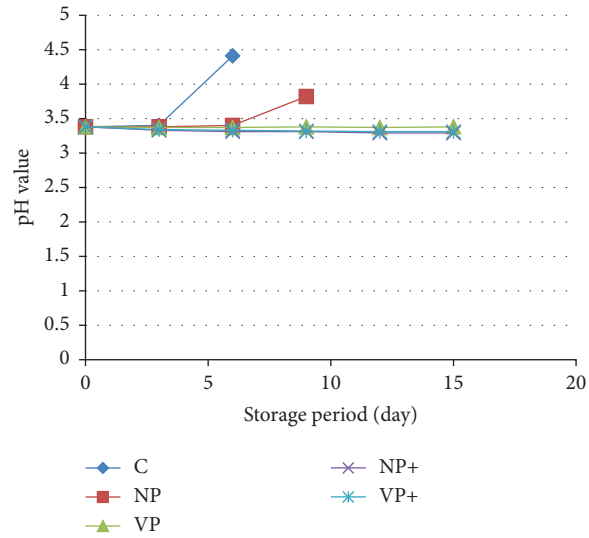

Figure 4: pH value of injera samples through different storage intervals. For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage, respectively, since the samples were spoiled.

4.2. 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 4, the pH values of the injera samples were found between 3.31 and 4.41.

The pH values of injera samples obtained in this study were comparable with the value reported in other related studies. According to Attuquayefio [35]; the result of the analysis of the different brands of injera was between 3.65 and 4.02. Also, the result reported from Ashagrie and Abate [6] 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 the 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 [35]. 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 the high acidic product and the high acidity of injera could account for the few numbers and types of organisms associated with its spoilage.

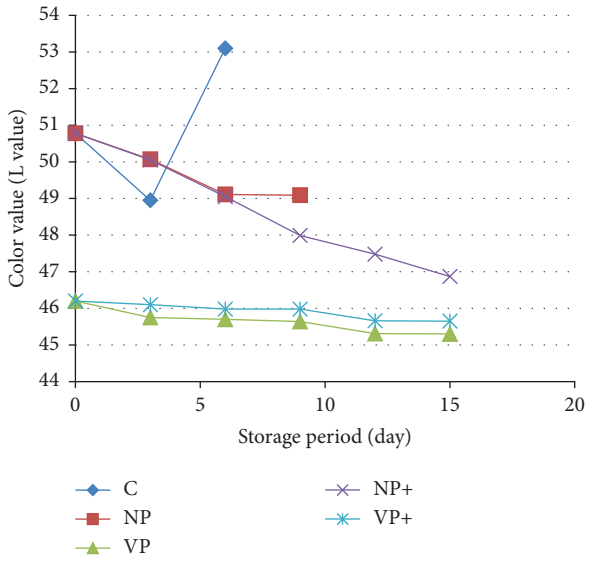

Figure 5: Colour (L value) of injera at different storage intervals. For "C" and "NP" treatments, the experiment was terminated at 6th and 9th day of storage, respectively, since the samples were spoiled.

4.3. Color. The superficial appearance and color of food are the first parameters of quality evaluated by consumers and are thus critical factors for the 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 [36].

Color of injera samples as detected by an electronic spectrophotometer (Konica Minolita Cm-600d Spectrophotometer) are presented in Figure 5. 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.

As shown 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 be 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 color (L^*) value of the samples whereas the 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 nonvacuum packed samples (NP&NP+) and control samples (C), respectively. This may be due to the 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 is stacked out or collapsed it may loss its shiny appearance or its lightness. These may be the reasons for the color 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 Cherie et al. [37]; 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 the 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 the 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 the variety of teff used has also its own impact. The result reported by Bhol and Bosco [38] showed that, L^* value estimation by color flex for control sample (wheat flour bread) was obtained at 75.24, which is agreeable with the result found in this study; since it is obvious that the color of refined wheat bread is more whitish or lighter than injera (had L^* value of 50.78 at the first day of analysis).

Table 1: Storage duration of injera without visible mold growth.

Treatments	Date of observation for the first visible mold growth	Storage duration (days)
C	6th day	4-5
VP	No mold growth until 15th day	More than 15
NP	9th day	7-8
VP+	No mold growth until 15th day	More than 15
NP+	No mold growth until 15th day	More than 15

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 the day of baking to the moment in which the samples of a batch presented any visible signs of molding (Table 1).

The results obtained from this study indicated that control samples were started to show visible mold growth on the 4th-5th day of storage. Whereas the number of visible mold singe-free days was prolonged to more than 15 days for the samples with the addition of preservative weather or not packed under vacuum (VP+ and NP+). Also, it was extended to 7-8 days for the nonvacuum packed sample with no addition of preservative (NP), and almost doubled when it is packed under vacuum

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

(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 6).

Commonly the expected visible mold sign-free storage period of injera under unpreserved condition is 3-4 days [6], 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 days) 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 [6]; 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% [39]. Thus, to achieve a significant shelf life extension, the elimination of O_2 from the package should be fast and complete [40]. Under good vacuum conditions, oxygen in the package headspace is reduced to <1%, i.e., levels which delay mold growth [13]. In this case, vacuum packaging becomes a preferable packaging method for the retardation of mold growth and extension of the 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 2).

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 [41]. 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 [41]. In the current study, a panel of 12 judges was described their degree of sensory acceptance to the injera samples with respective treatments. The color 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 a higher degree of liking. Appearance is another important factor which refers to the quality of the eyes (cells) of the honeycomblike structure of the top surface of injera formed during cooking due to escaping CO_2 bubbles [42] 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 the least degree of liking in terms of appearance and texture that indicates this method affects the 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 the overall acceptability test with a mean score of 1.75, which forced them to be at the very top of the Duncan posthoc 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 [13] as happened in this experiment too (Figure 7). 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.

Based on the average mean score of overall acceptability, nonvacuum 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, nonvacuum packed injera samples, especially the one

Figure 7: Pictures display a crumpling effect of vacuum packaging, via physical look or appearance of vacuum packed (VP) vs. nonvacuum packed (NP) injera samples. (a) Vacuum packed sample (VP) packed look. (b) Nonvacuum packed sample (NP) packed look. (c) Vacuum packed sample (VP) open look. (d) Nonvacuum packed sample (NP) open look.

without additional preservative sodium benzoate (NP) got a high preference which is compatible with the control sample. This proved that nonvacuum packaging is a preferable method than vacuum packaging for the objective of retaining the sensory quality of the selected product.

5. 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, the 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 the 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+ (nonvacuum packaging with preservative sodium benzoate) treatments. Also, the storage duration was extended to 7-8 days with NP (nonvacuum 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, nonvacuum packaging was found to be an 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 the 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 an effective method in mold growth retardation but it was least effective regarding staling and sensory acceptability. Thus using of nonvacuum packaging method together with the 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 [43–47].

Data Availability

All the data generated from this study have been presented in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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