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Food Safety Recommendation for Traditional Fermented Food by Small-Scale Producers: Injera as a Case Study

ABSTRACT

Injera is a traditional fermented food originally from Ethiopia, that has gained popularity in western countries. Injera is a fermented pancake-like bread prepared primarily with teff flour, but other cereals like wheat, barley, sorghum, maize flour can be used. Production of injera involves two rounds of spontaneous fermentation lasting 1 to 7 days. While there are no documented foodborne outbreaks associated with injera in the United States, injera may pose a food safety risk given the long fermentation process, and does not provide evident safeguards against the growth of microbial pathogens. From a public health point of view, there is an urgent need for food safety guidance specifically for small-scale producers as food systems are rapidly diversifying. The work creates food safety parameters that can be used by small-scale and household injera producers. In this review, the preparation of injera was summarized, along with the identification of critical control step and control measures to ensure safe production of the product.

INTRODUCTION

Fermentation is a process that has been used to preserve foods since the start of civilization. Nearly all food commodities can be fermented including meat, fish, milk, grains, fruits, and vegetables *(1)*. This preservation technique transforms the sensory characteristics, and nutritional content of foods. Fermented products highlight diversity of foods across the globe, driven by the availability and accessibility of raw ingredients as well as environmental conditions. Ethiopia is no exception, as it is home to a variety of fermented products including *tella* (fermented grain-based beverages), *cheka* (fermented cereals), *tej* (homemade wine), and injera, which is probably the most popular *(17)*. Injera is a traditional staple food consumed in all the parts of Ethiopia and Eritrea and some part of Somalia *(19)*. It is a pancake-like, circular, soft-textured, spongy, and resilient bread, approximately 6 mm thick with a 60 cm diameter. Teff is the common grain used to prepare traditional injera, but other grains like wheat, corn, sorghum, barley can be used *(19)*. The ratio of teff flour and other grains in injera can vary depending on traditional practices, previous

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experience, and recipes followed. The chemical composition of teff flour varies widely depending on the environmental conditions, soils and fertilizers used. Studies have shown that teff is predominantly starchy (73%) and contains minerals including iron, calcium, magnesium, and zinc compared to other flours like maize, brown rice and sorghum and millet. It is also rich with essential amino acids such as leucine, valine, proline, and glutamic and aspartic acids *(10, 19)*.

Injera has been gaining popularity in North America and European countries, due in part to the rise of Ethiopian immigrant populations increasing exposure of the broader public to traditional foods. Also, teff flour is gluten-free, which is a welcome alternative to wheat *(32)*. Furthermore, young consumers are demanding fermented foods beyond the traditional pickles, cheese, beer, and wine *(23)*. Given the growing market, it is reasonable to assume that many of the Ethiopian business owners will be classified as small and very small business under the Food Safety Modernization Act (FSMA). Under FSMA, a business that produces fermented foods to be sold in the United States, (U.S.) including injera, may be required to conduct a hazard analysis. Therefore, there is a need for more detailed assessment of the preparation, handling, and storage of injera using a hazard analysis critical control points based (HACCP) approach. Therefore, the overall goal of this document is to provide recommendations for the safe production of injera. A unique and collaborative approach was used to create the guidance document for safe manufacturing of injera. First, we partnered with a local Ethiopian restaurant owner in West Harlem, New York City. The regular conversations provided valuable input not only about injera production but also the cultural context. While the social and cultural patterns vary from one producer to another, these interactions helped make these recommendations accessible and acceptable to the targeted population. Second, peer-reviewed publications, blogs and other social platforms were reviewed to summarize recipes and develop the hazard analysis to determine critical control of points and control measures for the safe production of injera during fermentation and storage. The information provides pertinent safety recommendation for the production of injera, focusing on small-scale and household production.

Preparation and microbial composition

To prepare injera, teff flour is mixed with water and a starter culture (*ersho*) from a previous batch as summarized in *Figure 1*. This process, known as backslopping, is commonly used in preparation of traditional foods. The batter is then carefully mixed by hand or with utensils to form a thin and runny paste. The paste is initially fermented for 24 to 72 hours at room temperature *(2)*. After the first fermentation, a portion of the dough is cooked to a boil with water to form the *"absit."* This is the gelatinization process where the starch is hydrated producing a gel like texture and creating bubbles (eyes) *(29)*. The cooking process breaks the starch, producing simple sugar which are additional foods for microbes in the batter. The *absit* is then cooled down and added back to the original fermentation batter (from the primary fermentation). This step initiates the secondary fermentation lasting from 2 to 24 hours. The addition of the *absit* is critical for production, as it helps develop the unique texture and consistency, that leads to gas formation and rising of the dough *(25, 33)*. The addition of the *absit* leads to gas formation and causes the paste to rise. Without this addition, injera is powdery and has fewer eyes (holes in the pancakes), and is not appreciated by the consumers *(19)*. Finally, the injera batter is poured on a hot, oiled griddle, known as *mitad* and cooked for about 2 to 3 minutes. The cooking temperature of the *mitad* varies between 90 to 95˚C *(14)*. Once it is cooked, the finished injera can be stored for up to three days at room temperature in a traditional straw basket called *messob,* or stored under refrigeration until there are visible signs of spoilage including mold growth, discoloration, slimy texture, unusual odor, etc.

While there is limited food microbiology research on the injera process, there are several peer-reviewed publications on the topic. In one study, teff flour was sampled from 10 different households. Results showed that all the flour had at least 4 log CFU/g of microorganisms with total aerobic plate count, and seven samples had 3 log CFU/g of mold, suggesting that the teff flour can be high in microbial load *(3)*. Many studies have reported the predominance and importance of lactic acid bacteria (LAB) during the fermentation process. A study by Tadesse et al, aimed to identify yeasts present during injera fermentation using Inter Transcribed Spacer (ITS) sequencing. In that study, 97 samples of teff dough were collected and screened. Results identified yeasts belonging to the genera *Candida, Kazachstania, Pichia,* and *Saccharomyces (27)*. Another publication by Desiye and Abegaz sampled 34 samples over a 96-hour fermentation and collected samples at 6-hour intervals. A total of 107 lactic acid bacteria were identified including *Pediococcus pentosaceus, Lactobacillus fermentum,* and *Lactococcus piscium,* along with 68 yeasts such as S*accharomyces cerevisiae, Candida humilis, Candida tropicalis,* and *Pichia norvegensis (8)*. A similar study aimed to identify the dominant LAB species and yeast for the optimization of traditional injera dough. The research found five dominant LAB species (*Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus plantarum, Bacillus subtilus, Enterococcus casseliflavu*) and three dominant yeast strains (*Saccharomyces cerevisiae, Candida krusei, Pichia kudriavzevii*) *(28)*. In one another study by Ashenafi et al., 40 *ersho* samples were collected from four different households over 10 different sampling days *(3)*. The pH of all the ersho samples was consistently around 3.5, but

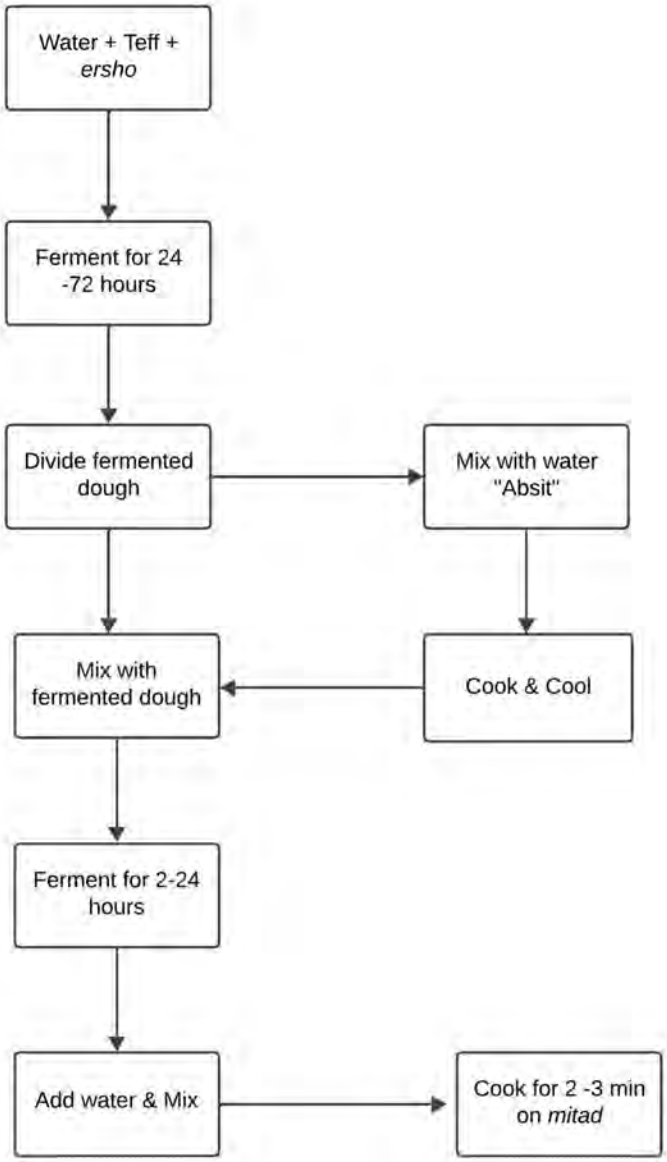

Figure 1. Flow chart for injera preparation.

the microbial population in the starter culture was highly diverse. *Bacillus* spores were detected along with five different species of yeasts: *Candida milleri, Rhodotorula mucilaginosa, Kluyveromyces marxianus, Pichia naganishii,* and *Debaryomyces hansenii,* which were detected at various concentrations in the four households *(3)*.

Potential food safety risks

To date, there are no known foodborne outbreaks specifically associated with injera. This might be because injera is considered a low-risk food, consumption is limited to niche, small markets, and/or because illnesses go unreported. However, several outbreaks in the U.S. have been linked to Ethiopian restaurants, though the specific food source was never identified. In March 2013, an Ethiopian restaurant was cited for five food safety violations, including improper handwashing, unsanitary equipment, and risk of cross-contamination, which may have contributed to the outbreak. *E. coli* was the pathogen of concern leading to this outbreak *(7)*. In March 2024, an outbreak was traced to a restaurant in Seattle where the illnesses symptoms were suggestive of *Clostridium botulinum* or *Bacillus cereus (15)*. While no outbreaks have specifically been linked to injera, small restaurants are known to receive significantly higher violations regarding their food safety culture or practices *(11, 16)*. Common risk factors include: 1) food from unsafe sources; 2) inadequate

cooking; 3) contaminated equipment; 4) improper holding temperatures; and 5) poor personal hygiene *(11, 31)*. These violations are often not intentional but highlight the need for proper formal food safety training as food safety requirements and good manufacturing practices may not be understood or correctly followed, especially in the presence of language barriers and other factors *(20, 21)*. Furthermore, the process of identifying relevant hazards and developing a food safety plan is challenging for small producers with limited scientific background. Nevertheless, the production of injera must be carefully evaluated because its complex fermentation process, if not properly monitored, may lead to foodborne outbreaks.

INJERA HAZARDS ANALYSIS

Injera producers must implement a food safety plan that evaluates the risk of physical, chemical and biological hazards. This document does not focus on chemical and physical hazards, nor does it address mold-producing toxin or mycotoxin contamination and biogenic amines *(13)*. Instead, it focuses on the biological hazards and critical control points (CCP) of the fermentation process and outlines preventive measures to be applied *(Table 1)*. These recommendations are based on HACCP principles and the implementation of Good Manufacturing Practices (GMP) to ensure the safety of small-scale fermented foods, using injera as the model.

Step 1: Ingredient receipt and storage

The quality and safety of the raw material and water must be considered. Cereals such as teff flour have historically been found to be contaminated with pathogens such as *E. coli, S. enterica, Listeria monocytogenes,* and *B. cereus (4, 22)*. Teff flour is primarily contaminated with gram-positive organisms, specifically *Bacillus* spp. Some of these microorganisms are essential for the beginning of fermentation until *Enterobacteriaceae* reach a high enough concentration, which typically happens around 12 hours *(3)*. Additionally, the flour might be contaminated with a variety of soil microbes and fecal matter *(9, 35)*. Therefore, producers must request a certificate of analysis (COA) from their suppliers documenting that biological hazards have been eliminated or prevented according to regulatory needs (21 CFR Part 117). If a COA cannot be obtained, it is recommended that producers send each flour batch to be tested for pathogens of concern, according to their internal Food Safety Plan, like *Salmonella* spp., *B. cereus, E. coli* O157:H7. Manufacturers can also follow the guidelines by Sperber et al. for wheat flour where the threshold is <100,000 CFU/g for total (CFU: Colony Forming Unit) and <5,000 CFU/g for mold *(24)*. Finally, flour must be stored the stored at room temperature, covered and protected from environmental contamination, until it is ready to be used for production.

Monitoring the water quality is also critical to ensure the safe preparation of injera. The use of contaminated water can introduce undesirable microorganisms in the dough, which can negatively impact the fermentation process essential to achieving the texture and flavor of the final product. In sourdough bread, for example, poor water quality results in reduced fermentation efficiency and suboptimal bread quality *(26)*. Given these risks, it is imperative that water used in the preparation of injera be regularly monitored. In the United States, small-scale processors often operate in their household or in commercial or shared kitchen spaces. In such cases, water quality is either monitored at the municipal level or is incorporated into the facility's Current Good Manufacturing Practices (cGMPs), ensuring adherence to sanitary standards and the prevention of contamination. However, if processors utilize well water, it is advisable that they submit water samples to a certified laboratory for microbial analysis on a quarterly basis.

Step 2: Mix flour and water

The exact amount of the *ersho* (starter culture), as well as the flour-to-water ratio, are not standardized. As mentioned earlier, the preparation methods and cooking techniques vary based on family traditions and life experience *(19)*. *Ersho* contains a large number of microorganisms that accelerate the fermentation process. The most traditional technique is backslopping, which is very common in injera preparation. Because the process of adding culture starter is not fully controlled, using the same *ersho* over time may vary the quality of the product and can result in pathogen contamination. One possible way to avoid inconsistency and ensure safety is to purchase commercially available *ersho*. Unfortunately, at the time of this writing, there isn't a commercially available *ersho* for purchase on the market. Another option is to regularly test the starter culture for pathogens. This option comes with its own challenge, given the high laboratory test costs and delays in production due to waiting for results. Alternatively, manufacturers can prepare fresh *ersho* 36 to 48 hours prior to injera preparation. This option, though time-consuming and labor-intensive, minimizes the risk of contamination and pathogen growth. After mixing the flour, water and *ersho* to create the initial batter, it must be covered and protected from environmental contamination during the fermentation process.

Step 3: Ferment for 24–72 hours–CCP

Once water and flour, with or without starter culture, are combined, the dough is stored at room temperature to initiate fermentation. The fermentation step is a CCP because growth of pathogenic microorganisms can be controlled by monitoring the pH and temperature. Previous studies have monitored the pH of the batter during injera fermentation. Nigatu et al. evaluated different combinations of flour and water ratio for the preparation of injera *(34)*. In all those

TABLE 1. Food safety control steps during injera preparation

Steps	Hazards created, eliminated or reduced	Preventive measures	CCP
1. Ingredient receipt and storage		• Supplier verification: Certificate of Analysis for teff or other flour used • Store flour in a dry (if possible cool) area with limited storage time.	Yes
2. Mix flour and water 3. Ferment for 24–72 hours (starter culture)	Physical: Insects, stones, dirt, glass Biological: Contamination with *L. monocytogenes, Bacillus* spp., *Clostridium* spp., *S. aureus*, *Salmonella* spp.; Growth of microbial pathogens	• Run flour through sieve/strainer and discard any visible foreign objects • Clean fermentation containers to reduce contamination. Discard any damaged containers. • Mix flour and water as desired. **RECORD** initial pH and cover containers to protect from environmental contamination. • If pH does not drop after 24 h (with starter culture) or at 48 h (without starter culture), discard product. • At the end of first fermentation, the final pH should be < 4. If not, discard product.	Yes
4. Divide fermented dough 5. Add hot water or cook	Biological: Survival of *B. cereus* spores	• Add boiling water to part of the dough and mix or cook as desired.	Yes
6. Add remaining fermented dough and mix to desired consistency. Add water as needed	Biological: Contamination with *L. monocytogenes*	• Measure and record pH; pH should be <4. • Check temperature, it must be below <70°F/21°C within 2 hours.	No
7. Ferment for 2 to 24 hours	Biological: Survival of *B. cereus* spores	• After fermentation is complete, check pH. pH must be below 4. If not, discard product.	Yes
8. Discard excess water as desired and cook		• Check mitad (griddle) temperature to be 165°F or above. Cook for at least 15 seconds as desired. • Consume shortly.	Yes
9. Packaging and storage		• Store in clean food grade container at room temperature for up to 3 days or in the refrigerator at <41°F. • Discard samples if there are any visible signs of mold.	No

variables, the pH dropped from 5 to 4.2 in 96 hours. Another study evaluated the pH change during the injera primary fermentation and found that the pH dropped below 4 within 12 hours of fermentation when using 1:3 flour-to-water ratio, and it remained below 4.0 pH for 72 hours *(5)*. This information suggests the different ratio of flour-to-water does not affect the pH at the end of the injera primary fermentation, but rather the time it takes to achieve the final pH.

Therefore, a maximum pH of 4 was established because it would control for growth of pathogens such as *E. coli* O157:H7, *Salmonella* spp., *B. cereus,* and *S. aureus,* based on the pH and water activity (Aw) of injera as per the Hazard Analysis and Risk Based Preventive Controls for Human Food: Guidance for Industry Document *(30)*. This pH value inhibits the growth of pathogens especially in the event the flour is contaminated *(18)*. Therefore, producers must measure the initial pH immediately after the batter is mixed and after 24 hours. If a starter culture is used, the pH must be equal to or below 4 within 24 hours. Without a starter, the pH must drop to or below 4.20 within 48 hours. If the pH value does not drop to or below 4 within 48 hours, the teff batter must be discarded as this indicates that fermentation is either not occurring or proceeding at a sufficient rate to inhibit pathogenic growth. Storage temperature is also critical during injera fermentation and must be monitored. Ambient temperature ranges between 20–25˚C in North America (U.S. and Canada). However, in Ethiopia and other parts of the world where injera is traditionally prepared, room temperature is several degrees higher ranging from 28 to 50°C depending on the season *(12)*. Given that temperature affects the rate of fermentation, producers should monitor the pH and temperature by acquiring a cleaned and sanitized food-grade thermometer, knowing that warmer temperature will speed up the process. More importantly, the pH should be monitored before, after 24 hours, and at the end of fermentation to ensure limiting conditions for pathogen growth.

Steps 4, 5, and 6: Divide fermented dough, add water and/or cook, mix with remaining dough

Once fermentation is complete, the liquid on top is discarded and what remains is a very thick batter. A portion of the dough is divided and mixed with water to create the *absit* which the gelatinization process *(29)*. The *absit* must be cooked to at least 165°F (74°C) for 15 seconds to inactivate potential pathogens *(6)*. While the cooking step eliminates the vegetative cells, there is a likelihood that spores, if present, can germinate in the mixture. However, since the final pH after that cooking step remains below 4, pathogens including *B. cereus,* and *C. botulinum* are inhibited from growing. After the cooking step, the *absit* is mixed back into the original fermented dough and water is added as desired. The newly mixed batter must be monitored during cooling, reaching 70°F (21°C) within 2 hours *(31)*. Provided the person preparing the injera follows GMPs, these steps pose a low microbial risk.

Step 7: Ferment for 2–24 hours

After the dough is mixed, it is fermented again for at least 2–24 hours. Similarly to what is described in Step 3, both temperature and pH must be monitored. The pH must be checked and be below 4 throughout the fermentation process.

Step 8: Discard excess water and cook

After fermentation, the excess water is discarded, and the dough is mixed with additional water to the desired consistency. The final batter is poured on a *mitad* or hot plate and cooked for about 2–3 minutes. Provided that the cooking vessel temperature is hot enough to hold the batter at a minimum of 165°F (74°C) for 15 seconds, any potential pathogens that may have contaminated the final product will be controlled here. Afterwards, injera can be consumed immediately. If not, it can be stored at room temperature for 3 days maximum or refrigerated, covered and protected from environmental contamination. It can also be vacuum packed for shelf-life extensions.

CONCLUSIONS

This provides a realistic approach to safe production of injera considering the technical, and safety concerns as well as cultural information gathered through conversation with our partner. This helps identify critical food safety parameters and feasible ways to start addressing them. Given that injera is currently prepared on a smaller scale (home, in a restaurant, or a shared kitchen), the steps provided in this paper can be used as educational resources for safe production of injera. More studies are needed on the survival of pathogens during injera preparation under defined conditions, especially in small-scale and household conditions.

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