



WERABE UNIVERSITY

**COLLEGE OF NATURAL AND COMPUTATIONAL
SCIENCES**

DEPARTMENT OF BIOLOGY

**MICROBIAL QUALITY, SAFETY AND ANTIMICROBIAL RESISTANCE
OF PATHOGENS ISOLATED FROM KOCHO AND BULLA IN ALICHO
WURIRO WOREDA, SILTE ZONE, ETHIOPIA**

THESIS REPORT SUBMITTED TO DEPARTMENT OF BIOLOGY IN PARTIAL
FULFILMENT OF THE AWARDS FOR MASTER OF SCIENCE DEGREE IN
MICROBIOLOGY (M.SC)

By

ALIMA HALIL

**FEBRUARY, 2026
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Declaration

I hereby declare that this thesis is my own work and that all sources have been acknowledged. No part of this thesis has been submitted for any degree or qualification at any other university. I have complied with the ethical guidelines and obtained all necessary permissions for data usage where applicable.

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Approved by:

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Signature

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List of Acronyms and Abbreviation

AWWAO	Alicho Wuriro Woreda Agricultural Office
ANOVA	Analysis of Variance
AWWTO	Alicho Wuriro Woreda Transport Office
CV	Coefficient of Variation
CFU/mL	Colony Forming Units per Milliliter
MASL	Meters Above Sea Level
SPSS	Statistical Package for Social Sciences
TAMBC	Total Aerobic Mesophilic Bacterial Count
TCC	Total Coliform Count
Y and MC	Yeast and Mould Count

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Abstract

*Enset (Ensete ventricosum) is a staple crop in central Ethiopia, mainly consumed as kocho and bulla. Kocho is produced by fermentation, while bulla is a starchy by-product of higher quality. Fermentation improves preservation and nutritional value, driven by microbial activity, particularly staphylococci. However, little is known about pathogen presence and antimicrobial resistance in enset foods. This study assesses the microbial load of kocho and bulla to address food safety concerns in Alichu Wuriro Woreda. Data were gathered through household surveys, where structured questionnaires captured sociodemographic details as well as information on traditional processing and Underground storage methods. Most households in the study area were small to medium, with 72.2% having 1–5 members. The majority of respondents were middle-aged (36–45 years, 52.8%), predominantly female (62.0%), and married (65.7%). Education was limited, with 48.1% reaching the read and write level and 27.8% illiterate, while all respondents were farmers, reflecting an agriculture-based community. Fermented kocho was mainly stored 7–12 months or longer, requiring 1–2 months before consumption. All households used underground pits, with leaves changed every 2–3 weeks and pits every 45–60 days to maintain quality. Microbial analysis of enset foods showed that Kocho had higher bacterial contamination, with total coliform and Enterobacteriaceae counts of $5.3 \pm 0.6 \log \text{ CFU/g}$, compared to $4.7\text{--}4.8 \log \text{ CFU/g}$ in Bulla. Conversely, Bulla exhibited higher yeast and mold counts ($5.6 \pm 0.4 \log \text{ CFU/g}$) than Kocho ($4.9 \pm 0.8 \log \text{ CFU/g}$). Staphylococci and total aerobic mesophilic bacteria were similar in both products, indicating Kocho favors bacterial growth, while Bulla supports fungal proliferation. Kocho and Bulla were contaminated with *Staphylococcus aureus* and *Salmonella*. Kocho showed higher contamination (44.4% *S. aureus*, 7.4% *Salmonella*) than Bulla (35.1% *S. aureus*, 5.5% *Salmonella*). Overall, 39.8% of samples had *S. aureus* and 6.5% had *Salmonella*, indicating both products pose potential public health risks. The findings highlight food safety concerns associated with traditional processing and long-term pit fermentation practices. It is recommended to improve hygiene practices during the processing, handling, and storage of kocho and bulla.*

Keywords: Bulla, Drug resistances, *Ensete ventricosum*, Kocho, Pathogen,

1. INTRODUCTION

1.1. Background of the Study

Enset offers food, fiber, animal feed, and medication year-round (Brihanu, 2015). Kocho, bulla, and amicho are the principal food types that can be obtained from enset (Yemataw *et al.*, 2013). The starch that has fermented, or kocho, is made from grated corms and decorticated (scraped) leaf sheaths. Bulla, a starchy liquid, is obtained by grating corms and scraping off leaf sheaths. When the thick liquid dries, a white powder with high starch content is produced (Yemataw *et al.*, 2016).

The first application of biotechnology is fermentation, which uses the growth of microorganisms to convert raw materials into products with additional value (Tuffa *et al.*, 2017). As fermented foods are sold for preservation, safety, and sensory qualities in both industrialized and developing countries, it is significant in today's world. Traditional methods are used in Ethiopia to produce and consume fermented foods and drinks, such as tej, beer, and liquor (Jeong *et al.*, 2023).

Understanding the fermentation process is especially important for Ethiopia in order to properly utilize the crop. In pits lined with enset leaves, fermented kocho is frequently kept. According to Andeta *et al.*, (2018), the kocho can be kept for several months or even years in a storage pit, but it must be left there for at least a month. Women must spend a significant amount of time processing and preparing kocho. The corm must be graded and the leaf stalks removed in the initial step. After that, the pulp is crushed to release the starch, and the fibers are removed. This is placed in an approximately 1.5 m deep and 1 m wide pit, sealed with enset leaves, and then tightly crammed with stones. After that, it is allowed to ferment, a process that could take four months to a year (Daniel & Chung, 2020).

The harvesting season, fermentation duration, clone type, processing methods, and age of the harvested enset plant all affect the quality of the kocho (Mulatu, 2021). It is said that the quality improves with longer fermentation times when stored in a pit for years in cooler climates (Andeta *et al.*, 2019). Reported that fermentation occurs quickly in warmer climates and ends in one to two months. But because of the differences in variety selection, fermentation time, and

processing technique, kocho is subject to numerous limitations that affect quality attributes. Furthermore, which parts of the corm and leaf sheath are processed affects the quality within a single plant (Bosha *et al.*, 2016).

During processing, bulla a tiny amount of starchy product that is insoluble in water can be separated from kocho by pressing and decanting liquid. Bulla can be cooked straight through without going through a fermentation process, or it can be allowed to dry and ferment like kocho. Primarily composed of fully mature enset plants, it is regarded as the highest-grade enset food available (Tefera *et al.*., 2019). The Alichu and other central Ethiopian ethnic groups largely consume kocho and other enset food products as staple foods. In contemporary urban environments, enset foods are growing in popularity across all ethnic groups. Cheese, kitfo (spicy ground meat), and kocho are now served at weddings, specialty restaurants, and holidays (Tuffa *et al.*, 2017).

Potential Issues with Enset Products Nutritional Limitations Enset itself is low in protein and certain essential nutrients. Relying solely on enset products can result in nutritional deficiencies if not complemented with other food sources (Papini, 2018). Incomplete processing the traditional processing methods involve fermenting the enset, which helps break down toxic compounds. If not done correctly, consumption of inadequately processed enset can lead to health risks (Bosha *et al.*, 2016). **Bacterial Contamination** Like any agricultural product, enset can become contaminated with harmful bacteria if proper hygiene standards are not followed during cultivation, harvesting, and processing (Pandey, & Savita 2017). **Pesticide Residue** if pesticides or herbicides are used during the cultivation of enset, residues may remain on the plant, posing a risk to human health upon consumption (Awulachew, 2021). **Cultural and Economic Impact** a heavy reliance on enset can make communities vulnerable to climate-related shocks. If enset production is impacted due to drought or disease, food insecurity can arise (Awulachew, 2021).

Microorganisms can exist in various forms, including bacteria, yeasts, and molds. In the context of fermented products like kocho and bulla (derived from *Ensete ventricosum*), these microorganisms play crucial roles. **Beneficial Microorganisms** Yeasts these microorganisms also aid in fermentation, contributing to flavor and texture (Alam *et al.*, 2024). **Pathogenic**

Microorganisms Some microorganisms, such as certain strains of *Staphylococcus aureus* and *Salmonella*, can be harmful and cause foodborne illnesses if proper hygienic practices are not followed during the processing of kocho and bulla (Mulatu, 2021).

The quality of kocho is influenced by the variety of enset plants, processing techniques, and fermentation times (Andeta *et al.*, 2019). Traditional food fermentation is greatly aided by microbes, which can also be used to increase and standardize the fermentation process, improve shelf-life, and improve nutritional quality and organoleptic characteristics (Andeta *et al.*, 2019). The fermentation process uses the dynamics of microbes to break down starch, protein, and fat, changing the food's overall nutritional and sensory quality (Andeta *et al.*, 2018). The *Staphylococci* is the predominant microbial group throughout the entire enset fermentation process (Awulachew, 2021). The fruits are brous, oblong-obovate, 8–15 cm × 3 - 4.5 cm, and turn orange when fully grown. This is because they can withstand an acidic environment (Fanta & Neela, 2019). The present study was conducted to evaluate the microbial load and safety of kocho and bulla samples obtained from Alicho Wuriro district, and to investigate the antimicrobial resistance patterns of *Staphylococcus aureus* and *Salmonella* isolates recovered from these samples.

1.2. Statement of the problem

Kocho and bulla, staple food products derived from *Enset ventricosum* (false banana), are widely consumed in Alichu Wuriro woreda of Ethiopia due to their cultural and nutritional importance. However, their production and processing are largely carried out using traditional, unhygienic, and unstandardized methods (Mulatu, 2021). Fermentation and storage often occur in pits or local containers under uncontrolled environmental conditions, creating favorable environments for microbial growth (Ayalew & Yeshitila, 2011). As a result, these products may harbor high microbial loads, including pathogenic and spoilage microorganisms, which compromise food safety and pose significant public health risks (Awulachew, 2021). Despite their importance to household food security and the local economy, there is limited scientific information on the microbial quality and safety of kocho and bulla in the district (Tsegaye & Struik, 2002).

It is therefore essential to quantify the total bacterial count, yeast and mold load, and detect specific pathogens such as *Staphylococcus aureus* and *Salmonella* in kocho and bulla samples collected from households in the district. Additionally, assessing the antimicrobial resistance profiles of bacterial *S. aureus*, *Salmonella* isolates.

1.3. Objectives

1.3.1. General Objective

The general objective of this study was to investigate the microbial quality, safety and antimicrobial resistance profile of some selected human pathogens and of kocho and bulla collected from Alichu Wuriro Woreda.

1.3.2. Specific Objectives

The specific objectives of this study were to

- Assess household sociodemographic characteristics that may influence processing, handling, and storage hygiene of enset-derived foods.
- Evaluate the bacterial and fungal load of kocho and bulla collected from producers.
- Determine the prevalence of common foodborne pathogens in kocho and bulla.
- Evaluate the antimicrobial susceptibility pattern of pathogens isolated from kocho and bulla.

2. REVIEW OF RELATED LITERATURE

2.1. Overview of Enset

One potentially useful native crop that can be grown anywhere in Ethiopia is enset, which has the potential to produce food. The study area also uses it as a cash crop and a staple food (Ayele & Sahu, 2014). Foods made from (*Enset Ventricosum*), such as bulla, kocho, and Amicho, are well-liked, but the kocho is most prevalent. Before the plant flowers, its pseudo stem and leaf midribs are cut. After the pulp has been scraped, it ferments for ten to fifteen days before being made into steam-baked flatbread (Tefera *et al.*, 2019). Approximately 7 million individuals eat low-protein Enset products as staples or co-staples; they frequently do so without the necessary protein supplement but occasionally only with foods high in vitamin A. Leaf, fiber, and other leftover materials are used to make ropes, food packaging, cattle feed, and building materials for homes (Papini, 2018).

The term "fermented kocho product" refers to kocho food that has either been fermented as a type of energy-producing microbial metabolism in which an organic substrate, typically a carbohydrate, is incompletely oxidized and an organic carbohydrate acts as the electron acceptor, or it has been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification to the food (Zorina & Obozova, 2011). In Ethiopia, kocho is a traditional fermented food product made by fermenting parts of the *Ensete venenosum*, or "false banana." It is made by scraping and fermenting the pseudo stem and corm using solid state fermentation (Awulachew, 2021).

2.1.1. Food uses of enset plant

The two main food items made from the enset plant are known locally as "bulla" and "kocho." Kocho is the ferment (Daniel & Haile, 2023). kocho requires extensive processing and preparation, which is done by women. Grading the corm and removing the leaf stalks constitute the initial step. After the fibers are separated, the pulp is crushed to release the starch. This is placed in a pit that is roughly 1.5 meters deep and 1 meter in diameter. It is then sealed with enset leaves and weighed down with stones. It is then allowed to ferment which requires weeks to months, which may last from 4 months to three years (Ayalew & Yeshitila, 2011). The pit is opened at intervals to allow aeration, and the enset leaves are replaced. This is repeated until the desired fermentation quality is reached or the food is needed. Finally, the fermented starch is dried and treated as flour. This can be used to prepare a pancake – like bread, which is eaten with

milk and cabbage. kocho is increasingly exported to urban markets; kocho can be stored for a long period of time without spoiling. The quality of Kocho depends on the age of the harvested enset plant, the type of clone (variety), and harvesting season. Moreover, within one plant, the quality is influenced by the part of leaf sheath and corm processed (Zewdie *et al.*, 2008).

Bulla: - is a water insoluble starchy food product obtained by squeezing the contents of the mixture of the decorticated leaf sheath and grated corm and then decanting the liquid part. Bulla ‘can be eaten as porridge or as flat bread. The local people in the study area reported bulla’ as the best quality and the most preferred enset-derived food product (Tsegaye & Struik, 2001).

2.1.2. Fermented Enset

Kocho is a traditionally fermented and indigenous starch-rich food product in Ethiopia that is prepared from enset plants (Tefera *et al.*, 2020). Next to injera and wheat bread, it is a popular fermented food consumed in Ethiopia (Tefera *et al.*, 2020). Scraped pseudo stem, pulverized corm, and decorticated enset pulp are mixed and fermented to produce carbohydrate-rich kocho (Yirmaga, 2013). It is widely consumed by millions of Ethiopians Olango *et al.*, (2014) and plays a crucial role in ensuring food security (Yirmaga, 2013). bread prepared from fermented enset is known as kocho bread Fanta & Neela, (2019) reported that kocho is rich in carbohydrates. However, it has low protein content (Bosha *et al.*, 2016). Kocho production requires a long period of fermentation which is prolonged from three to six months (Melaku *et al.*, 2024). quality of kocho food depends on the age of the harvested enset plant, accession type, Bekele *et al.*, (2023) harvesting season, and fermentation period (Weldemichael *et al.*, 2019). Moreover, within one plant, the quality is influence by the part of the leaf sheath and corm processed (Seid *et al.*, 2022).

2.2. Kocho

A mixture of the ground corm of the Enset plant and the scraped pulp of the pseudo stem is used to make the starchy food product known as kocho. *Enset Ventricosum* is a drought-resistant plant that can be grown as a substitute food source to help with the global food security issue. Therefore, it is an end product that was acquired from Enset for use in the research area and to generate revenue (Bekele *et al.*, 2023).

2.2.1 Traditional Kocho Fermentation.

Traditional kocho fermentation requires a variety of equipment and ingredients for processing as well as for accelerating its duration time. It is varied from place to place both in equipment used for processing and in ingredients required (Yirmaga, 2013). A step in the process of kocho fermentation is the collection of matured enset plants carried out by experienced women (Birmeta *et al.*, 2019). After a mature enset plant has been selected, leaves are removed, cleared and dried leaf sheaths from the plant, and the surrounding parts are prepared for the next steps (Blomme *et al.*, 2018). The dug-up underground corm is detached and cleaned to isolate the true stem from the root. The inner leaf sheaths are separated from the pseudo stem down to the real stem, which is a segment between the corm and the pseudo stem (Azera *et al.*, 2020). True stem is isolated from the underground corm (Jones *et al.*, 2013). Pseudo stem and corm of the enset plant are scraped and pulverized (Tsegaye & Struik, 2002).

Fermentation starts after kocho Bosh (Bosha *et al.*, 2016), is stored in an earthen pit, which may range from three months to one year for completion. It depends on the climatic conditions of that environment (Birmeta *et al.*, 2019). In warmer regions, fermentation is rapid and may terminate within 15 days to at least one month (Sahle *et al.*, 2018), while in the cooler regions, it is kept in a pit for years (Azera *et al.*, 2020). Traditional processing of enset has two phases: phase one (surface fermentation), the beginning of fermentation which is continued for about 15 days, and phase two (pit fermentation) (Andeta *et al.*, 2019). Kocho fermentation methods and times are different from location to location (Papini, 2018).

2.3. Bulla

The white-colored starch concentrate called bulla is made from scraped leaf sheaths, peduncles, and grated corms of the enset plant (Devant *et al.* 2016). By processing kocho further and removing some byproducts, bulla is a high-quality product that is produced. Bulla now commands a higher price on the market than kocho as a result. In the research area, it is thus one of the marketable cash crops that can be acquired from enset.

2.4. Medicinal use of enset plant

Ensete ventricosum is a very good indigenous food plant having great medicinal value in relation to infections that are caused during broken bone cases. The plant is well known for its use as setting broken bones. Farmers use enset plants as medicines not only for human beings but also for their animals (Awulachew, 2021). Products from enset are used in different forms in

traditional and modern medicine including expel of placenta, healing of bone fracture, anti-microbial activity against viral and bacterial. Different enset varieties were reported to have medicinal and religious (ritual) significance for the prevention, healing and other therapeutic purpose. According Vaou *et al.*,(2021)Toye is a variety with a light red pseudo stem and midrib with deep green leaf which grown in the Kaffa. The boiled corm and starch powder of this variety is eaten with milk to cure ailments such as joint and Toye are believed to have medicinal value and are used treat human and animals. The corm of enset is fed to stimulate placenta discharge and reduce delivery –associated stomachache and eaten with cheese butter and milk. Official anhydrates starch suspension and bulla is used together with milk for bone related problems (Bosha *et al.*, 2016).

2.4.1. Economic and Ecological Uses of Enset

Enset is a multipurpose crop that can be used for food, medicine, ornamental purposes, and animal feed. Farmers say that enset is their food, cloth, house, bed, cattle feed, and plate (Brihanu, 2015). The corm and pseudo stem are the main food sources (Fanta & Neela, 2019) . The main foods made from enset are kocho, bulla, and amicho Yemataw *et al.*,(2014), with bulla being made by squeezing out the starch-containing liquid from scraped leaf sheaths and grated corm and allowing the resulting starch to concentrate into a white powder. Amicho is cooked corm of enset plants, prepared and consumed in a similar manner to other root and tuber crops (Yemataw *et al.*, 2014).

Enset's capacity to produce food per unit of land and store processed goods like kocho for extended periods of time without going bad are two of its most notable traits (Yemataw *et al.*,2018). The by-product of enset that remains after decorating the leaf sheaths is enset fiber. Its strength is found to be comparable to that of Musa textiles, or abaca, an important crop for fiber (Zewdie *et al.*, 2008). Enset fiber is utilized in the production of mats, ropes, twines, and bags. The length and quality of the fiber are determined by the variety, age of the plant, and methods of extraction and story (Tesfaye & Girma,2017).

In a nation like Ethiopia, enset can make a major contribution to ensuring food security (Ayalew&Yeshitila, 2011). The refined enset product (kocho) yield ranged from 7 to 12 tons ha-1 year-1 on average. An average family of five to six people could have enough food from the 50–

60 enset plants that can be harvested each year (Pandey & Savita, 2017). Enset products can be kept in pits for extended periods of time without going bad and are available year-round as kocho.

2.5. Other uses

Considering enset agriculture is an organic farming system that solely uses farmyard manures and excludes the use of external chemical fertilizers, herbicides, and insecticides, it plays an important ecological role. Because enset is available during the years when drought is likely to occur, it is regarded as a safety-shield cattle feed. In home gardens, enset plantations function as windbreaks for enset and other crop nurseries. With vibrant ornamental landraces, endemic farming provides an aesthetic requirement for a household garden (Olango *et al.*, 2014).

2.6 microbial quality and safety of kocho and bulla

Microbial quality refers to the number and types of microorganisms present in food products, indicating their overall healthiness. This includes beneficial organisms that contribute to fermentation and flavor, as well as harmful pathogens that can cause foodborne illnesses (Finstad *et al.*, 2012). Safety relates to the assurance that food products, like kocho and bulla, do not contain harmful levels of pathogenic microorganisms (De Bruin *et al.*, 2016). It encompasses testing and evaluating potential risks, ensuring that the food is safe for consumption.

2.7 antimicrobial resistance pathogenic bacteria of kocho and bulla

Antimicrobial resistance (AMR) refers to the ability of microorganisms, such as pathogenic bacteria, to resist the effects of antimicrobial agents that once effectively eliminated or inhibited their growth. In the context of kocho and bulla, traditional Ethiopian food products derived from enset, WHO, (2020) AMR can pose significant health risks. Definition Pathogenic bacteria are harmful microorganisms that can cause diseases in humans, animals, and plants. In the production and processing of kocho and bulla, Arshad, & Lee, (2018) these bacteria may contaminate the food products, primarily through: soil, water, or improper handling practices can introduce pathogens during cultivation and processing.

2.7.1 Food Processing Techniques

Traditional fermentation processes may not always eliminate all pathogenic bacteria, leading to potential health risks if these bacteria develop resistance to common antimicrobial treatments. The presence of antimicrobial-resistant pathogenic bacteria in kocho and bulla can lead to Foodborne illnesses due to the consumption of contaminated products (Arshad, & Lee, 2018).

2.7.2 Public Health Implications

Difficulties in treating infections caused by resistant pathogens, leading to prolonged illness and increased healthcare costs. Understanding AMR in relation to kocho and bulla is crucial for ensuring food safety, protecting consumer health, and promoting sustainable agricultural practices (WHO, 2020).

2.8 Enset production capacity in Ethiopia more specifically in alicho weriro werada

Enset (*Ensete ventricosum*), commonly referred to as "false banana," is a staple food crop in Ethiopia, particularly in the southern and southwestern regions. Enset production capacity refers to the ability of Ethiopia to cultivate, harvest, and process enset plants for consumption and economic benefits(Andeta *et al.*, 2019). Cultivation the area of land dedicated to enset farming, influenced by factors such as soil type, climate, and agricultural practices (Andeta *et al.*, 2019). Yield The amount of enset that can be harvested per unit area, which varies based on land management, cultivation techniques, and plant varieties. Processing the methods used to convert harvested enset into edible products, such as kocho and bulla, highlighting the crop's importance in local diets and its role in food security (Tseday, & Jürgen 2022). Cultural and Economic Importance Enset not only serves as a food source but also plays a crucial role in the cultural identity and livelihoods of many communities in Ethiopia (Tseday, & Jürgen 2022).

3. MATERIALS AND METHODS

3.1. Description of the Study area

The study was conducted in Silte zone of Alichu Wuriro woreda. Alichu Wuriro woreda is one of the 15 woreda in the Silte zone, Central Ethiopia Region Ethiopia. The woreda is located 270 kilometers of the capital city of the Ethiopia (Addis Ababa). The woreda of the capital town is Kawakoto, which is located at a distance of 28 km from the Central of the Silte zone Werabe town Alichu Wuriro woreda transport office (AWWTO). These woredas are southern by wulberag woreda on the south, west by Misrak azernat berbare woreda on the west, and north Gurage zone (meskan werada) the eset by slit werada. The woreda covers a land area of 26,318 hectares Alichu Wuriro woreda agricultural office (AWWAO, 2015). The temperature 16-21°C and 2500-3400 masl. Alichu-Wuriro- woreda is one of the major crops production is the main economic activity in Alichu-Wuriro woreda about 5902.5 hectares land area covers by enset (AWWAO, 2015). A total of 28 kebeles are found in Alichu Wuriro Woreda, including Ado 2, Ageta, and Weshano.

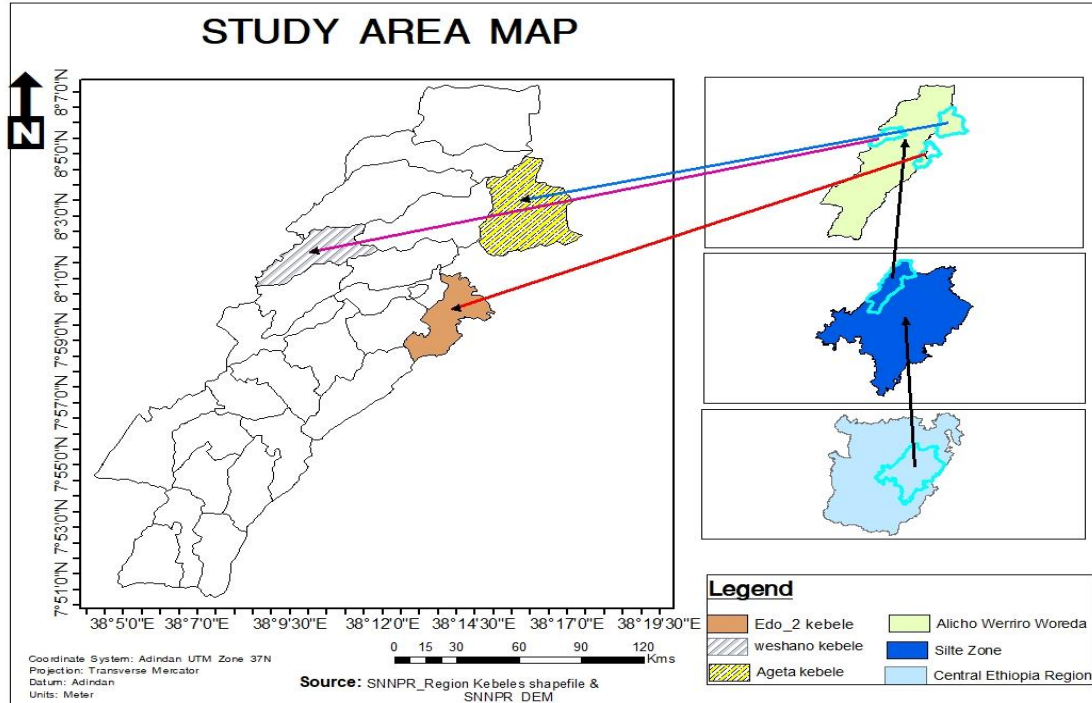


Fig 1. Geographical Map of Study Area

3.2. Study design

A cross-sectional study design was employed. Household surveys were conducted using structured questionnaires to collect sociodemographic information and document traditional processing and storage practices. Kocho and bulla samples were aseptically collected from participating households and transported under cold conditions to the laboratory.

3.3. Sample Size Determination and Sample Collection

According to data obtained from the Woreda Finance and Economic Development Office, the total population is estimated at 167,346, comprising 78,167 males and 89,179 females. The total number of households is 16,000, of which 12,500 are male-headed and 3,500 are female-headed.

From the study population, 108 households were purposely selected for sampling due to their high production of kocho and bulla, and the samples were collected from these households. The samples were obtained from processing pits at depths ranging from 5 to 25 cm. The samples, consisting of actively fermenting opaque kocho and bulla, were gathered from various household processing sites. The formula $Z^2 SD^2/e^2$ Charan & Biswas, (2013), was used to calculate the sample size of kocho and bulla. Where, $n = (1.96)^2 \times (0.153)^2 / (0.05)^2 = 36$ for this study. A total of 108 samples were collected, consisting of 54 kocho samples and 54 bulla samples from three study sites. The sample distribution was as follows: Weshano: 18 kocho and 18 bulla samples; Ageta: 18 kocho and 18 bulla samples; and Ado-2: 18 kocho and 18 bulla samples. Sampling was conducted to ensure representative coverage across the study sites and to capture variability in traditional processing and storage practices.

Each sample was immediately transferred into sterile tubes and securely sealed. Upon completion of collection, all samples were placed in sterile polythene bags and transported to the Werabe University Microbiology Laboratory and Werabe Comprehensive Specialized Hospital Laboratory. At the university laboratory, samples were stored at 4 °C until microbial analysis was performed.

3.4. Data collection

Since the study includes both survey and experimental components, the survey data were collected using structured questionnaires.

3.4.1 Microbiological analysis

The microbiological analysis was done through enumeration of total aerobic mesophilic bacterial count (TAMBC) total coliform count (TCC), Enterobacteriaceae count, *Staphylococci* count, Yeast and mold count, as well as by isolating and identifying microbial pathogens such as *S. aureus* and *Salmonella spp.*

3.4.2. Total aerobic mesophilic count

1.0 ml of the original sample appropriate dilution aliquot were spread-plated on Plate Count Agar (PCA) and then incubated at 32 °C for 48 h both samples before counting the colonies on countable plates (Adjlane *et al.*, 2014).

3.4.3. Total coliform count

A volume of 0.1 ml of the appropriate dilution aliquot was spread-plated in triplicate on Violet Red Bile Agar and incubated at 32 °C for 18 - 24 h. Colonies displaying purplish-red colors surrounded by reddish zones of precipitated bile were counted as total coliforms (De Bruin *et al.*, 2016).

3.4.4. Enterobacteriaceae count

Purple Colonies were counted on MacConkey agar as members of Enterobacteriaceae after incubated at 37 °C for 24-48 h. (Morshdy *et al.*, 2019).

3.4.5. Staphylococci count

The Staphylococci of kocho and bulla serial dilutions, 0.1 ml of the aliquot was spread plated onto Mannitol Salt Agar (MSA) (Oxoid) and incubated at 32 °C for 36 - 48 h (Bautista *et al.*, 2013).

3.4.6. Yeast and mold count

The Yeast and mold count of kocho and bulla sample were determined by direct plate count using Potato Dextrose Agar (PDA) supplemented with 0.1g chloramphenicol and incubated at ambient temperature for 2–5 days (Alam *et al.*, 2024).

3.5. Morphological identification and characterization

The morphology of the microbial cells was observed using Gislin *et al.*, (2018) methodology. Colony characteristics of the pure strains such as colony color, colony shape, and colony size were considered on agar plates to determine colony morphology. Microscopic examination of cell morphology and classification as rod and coccid, cell arrangement, motility (unidirectional

movement). The colonies were stained on a clean slide with biochemical stain with using normal saline, drop crystal violet, washing with water, drop iodine, wash and drop 95% ethyl alcohol, wash and finally drop safranin and observed under a light microscope using oil immersion objective (100 times) (Sumranjit & Chung, 2013).

3.6. Colonial characterization

The morphology of microbe isolates on solid medium was examined based on their cultural characteristics (colony shapes, size, pigment, and surface appearance).

KOH-test (test on lipopolysaccharide)

A colony was aseptically picked from the surface of plate count agar using an inoculating loop and stirred in the KOH solution for 10s to 2 min indicating the breakdown of the cell wall and release of cellular DNA negative and not release of cellular DNA positive. (Abdul-Hanan *et al.*, 2014)

Catalase test

The catalase test was conducted by flooding colonies with a 3% solution of hydrogen peroxide (H₂O₂) on a clean microscopic slide. Formation of bubbles indicated a positive test for catalase production and negative for not producing bubble (Biao *et al.*, 2019).

3.7. Detection of pathogens

3.7.1. Isolation of *Salmonella*

For the detection of *Salmonella* spp., 25 g of kocho and bulla samples were mixed with 225 ml of (buffer peptone water) BPW and incubated at 37°C for 24 h. Then 1ml pre-enrichment broth culture was added to 10 ml of selenite cysteine broth (Oxoid) and again incubated at 37°C for 24 h, thereafter, a loopful of suspension from a tube was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Oxoid). The presumptive *Salmonella* colonies (black colony surrounded by red color) were picked off, transferred to 5 ml nutrient broth (Oxoid), incubated at 37°C for 24 h, then streaked onto Nutrient Agar (Oxoid) for purity, and incubated at 37°C for 24 h (Ekateriniadou *et al.*, 2015). The presumptive *Salmonella* spp. was characterized by standard biochemical tests. The biochemical tests were done according to the procedure of Indole test-Methyl red test-voges-proskauer test-Citrate Utilization test –H₂S production test –Urease test (Finstad *et al.*, 2012).

3.7.2. Isolation of *Staphylococcus aureus*

For detection of *S. aureus* golden yellow colonies on MSA plates during *staphylococci* count both kocho and bulla were picked and transferred into 5 ml nutrient broth and incubated at 37 °C for 24 h for further purification. Then, a loopful of culture from the nutrient broth was streaked on nutrient agar supplemented with 0.75% NaCl and again incubated at 37 °C for 24 h. (Marino *et al.*, 2011). Gram-positive cocci with clustered arrangement under the microscope were subjected to preliminary biochemical tests: oxidase, catalase and coagulase tests (Sheehan, 2016).

Antimicrobial Susceptibility testing for the isolated pathogens

Antimicrobial susceptibility testing was investigated on Mueller Hinton Agar (Oxoid) plates following the standard disk diffusion techniques. The antibiotic discs were placed on the medium by using forceps and incubated at 37°C for 18 h and the zone of inhibition was measured manually with a transparent ruler. The results of the antimicrobial susceptibility were interpreted based on the guidance of National Committee for Clinical Laboratory Standards (Jones *et al.*, 2013). The following standard discs (Oxide) and their potency (µg/ml) were used depending up on the antibacterial spectrum, toxicity, effectiveness and availability (Isnard *et al.*, 2016): ampicillin (10), ciprofloxacin (5), gentamycin (10), (30), and tetracycline (30) were used for *Salmonella* spp. ciprofloxacin (5), gentamycin (10), penicillin G (10), and tetracycline (30) were used for *Staphylococcus aureus*

3.8. Statistical Data analysis

The percentage of coefficient of variation (% CV) was calculated to verify if there was significant variation in microbial counts within the kocho and bulla samples analyzed. The data obtained from the respondents were analyzed using the SPSS software version 21%. The Mean values of microbial counts isolated from kocho and bulla were compared using a one-way ANOVA and the significance of their differences were considered at 95% confidence interval ($P < 0.05$).

3.9. Ethical consideration

Ethical clearance was obtained from research review and ethical committee of postgraduate of Werabe University.

4. Results and Discussion

4.1. Socio-demographic characteristics of respondents

The household size of most respondents ranged between 1–5 members (72.2%), while about a quarter (26.9%) had 6–10 members. Only 0.9% reported having a family size of 11–15, showing that small to medium households are dominant in the study area. With regard to age, the majority of respondents were in the 36–45 age group (52.8%), followed by those aged 26–35 (16.7%) and 46–55 (16.7%). A smaller proportion belonged to the older group above 56 years (10.6%), while very few (3.7%) were between 15–25 years. This indicates that middle-aged household heads form the largest group of respondents. In terms of gender, 62.0% were female and 38.0% male, showing that women made up the majority of respondents. Regarding marital status, most were married (65.7%), while others were widowed (15.7%), unmarried (13.0%), and a small fraction were divorced (5.6%). Educationally, nearly half of the respondents had reached the Read and write level (48.1%), while 27.8% were illiterate. Smaller proportions had attended elementary school (17.6%) and high school (6.5%), suggesting that education beyond primary level was relatively limited. Occupationally, all respondents (100%) were farmers, confirming the agricultural basis of the community's livelihood (Table 1).

Table 1. Socio-demographic Characteristics of farmers.

Characteristics	Category	Number of respondents(n=108)	
		Frequency	Percent (%)
Family size	1 – 5	78	72.2
	6 – 10	29	26.9
	11 – 15	1	0.9
Age	15-25	4	3.7
	26-35	18	16.7
	36-45	57	52.8
	46-55	18	16.7
	>56	11	10.6
Gender	Male	41	38.0
	Female	67	62.0
Marital status	Married	71	65.7
	Unmarried	14	13.0
	Widowed	17	15.7
	Divorce	6	5.6
Education status	Read and write	52	48.1
	Elementary	19	17.6
	high school	7	6.5
	Illiterate	30	27.8
Occupation	Farmer	108	100.0

Fermentation techniques of Kocho and Bulla

Most respondents reported keeping fermented kocho in storage for 7 to 12 months (57.4%), while about 36.1% kept it for more than a year (Table 2). Only a small proportion (6.5%) stored it for 2 to 6 months, showing that longer storage is generally preferred. With regard to fermentation time before the kocho becomes fit for eating, nearly two-thirds (63.0%) of the respondents indicated that it takes 1 to 2 months, while 22.2% mentioned more than two months. Only 14.8% stated that it becomes ready within 2 to 4 weeks. This suggests that the majority consider a medium fermentation period optimal. In terms of storage technique, all respondents (100%) reported using underground pit storage, indicating that this is a universal practice in the

community. For the changing of wrapping leaves, about half of the respondents (50.9%) reported doing so every 15 to 25 days, while 28.7% changed them every 15 to 20 days. A smaller proportion (20.4%) extended the interval up to 15 to 30 days. This shows that most farmers prefer a 2- to 3-week interval to maintain quality. When it comes to changing the storage pits, the majority (59.3%) replaced them every 45 to 60 days, while 37.0% did so more frequently, every 30 to 35 days. Only 3.7% reported extending the interval up to 45 to 75 days, indicating that frequent pit replacement is a common practice (Table 2).

Table 2. Fermentation techniques of Kocho and Bulla from farmers.

Number of respondents(n=108)			
Characteristics	Category	Frequency	Percent
How long is fermented kocho kept in storage	2 to 6 months	7	6.5
	7 to 12 months	62	57.4
	More than one year	39	36.1
how long does it take for the kocho to ferment and become fit for eating	2 - 4 weeks	16	14.8
	1 - 2 months	68	63.0
	> than two months	24	22.2
Which storage technique do you use	Underground storage (pit storage)	108	100
Changing wrapping leaves Time interval	15 to 20 day	31	28.7
	15 to 25 day	55	50.9
	15 to 30 day	22	20.4
Changing storage pit Time interval	30 to 35 day	40	37.0
	45 to 60 day	64	59.3
	45 to 75 day	4	3.7

General management conditions of Kocho and Bulla

All respondents (100%) reported living in the Dega agro-ecological zone (Table 3). The primary reason for producing enset was for food (77.8%), followed by income generation (17.6%), and to a lesser extent for medicine (3.7%) and other purposes (0.9%). This shows that enset plays a central role as a staple food, with secondary importance for cash and traditional medicinal use. A large majority (90.7%) believed that enset is more crucial than other crops for ensuring a year-round food supply, while 9.3% disagreed. Similarly, 64.8% of respondents considered enset more important than other crops for cash income, though 35.2% did not share this view. Most respondents (88.9%) also perceived enset-based food to be better than other food types, while a smaller proportion (11.1%) preferred alternative (Table 3). Furthermore, all respondents (100%) agreed that enset distribution in their area was important. Regarding microbial load, the majority (85.2%) stated that microbial groups in kocho and bulla are presented during fermentation, while 14.8% indicated they appear following fermentation. On labor availability, all respondents (100%) agreed that family labor was sufficient for producing kocho and bulla. In terms of consumption, almost all (96.3%) reported that their families eat kocho and bulla, while only a few (3.7%) did not. Similarly, all respondents (100%) had experience using kocho and bulla products. When asked about safety, all respondents (100%) reported no problems with consumption of these products. However, in terms of frequency of use, responses were nearly evenly split: 49.1% always consumed kocho and bulla products, while 50.9% did not consume them consistently (Table 3).

Table 3. General management conditions of Kocho and Bulla Alichu wuriro werada

Characteristics	Number of respondents(n=108)		Percent (%)
	Category	Frequency	
the agricultural ecology	Dega	108	100
	Food	84	77.8
	income in	19	17.6
what is your main goal for producing Enset	cash		
	Medicine	4	3.7
do you think that enset is more crucial than other crops to ensuring a year-round supply of food	Other	1	0.9
	Yes	98	90.7
	No	10	9.3
do you believe that enset is more crucial to producing cash income than other crops	Yes	70	64.8
	No	38	35.2
do you think Enset food is better than other food types	Yes	96	88.9
	No	12	11.1
your opinions on the enset distribution in your area	Yes	108	100
when are the microbial load groups presented in Kocho and Bulla	During fermentation	92	85.2
	Following fermentation	16	14.8
Is the labor provided by your family sufficient for	In agreement	108	100
the production of kocho and bulla			
Does your family eat Kocho & Bulla	Yes	104	96.3
	No	4	3.7
Do you have any experience using Bulla and Kocho products	Yes	108	100
Do you think there is problem with consumption of Kocho and Bulla product	No	108	100
Do you always use Bulla and Kocho products	Yes	53	49.1
	No	55	50.9

Microbial counts of Kocho and Bulla

The microbial analysis revealed distinct differences between bulla and kocho. The mean total coliform count was higher in kocho (5.3 log CFU/g) than in bulla (4.7 log CFU/g), indicating a greater level of coliform contamination in Kocho. (Fig.2 and 3) Similarly, Enterobacteriaceae counts were elevated in kocho (5.3 log CFU/g) compared to bulla (4.8 log CFU/g), suggesting that kocho may be more prone to contamination during handling or fermentation. In contrast, yeast and mold counts were greater in bulla (5.6 log CFU/g) than in kocho (4.9 log CFU/g), which could be linked to the storage environment or differences in substrate composition that favor fungal growth. *Staphylococci* levels were nearly identical in both products (5.4 log CFU/g), and the total aerobic mesophilic bacterial counts were also comparable (4.8 log CFU/g in both), showing no significant variation between the two. Overall, kocho exhibited higher bacterial contamination, whereas bulla had higher fungal loads. (Fig.2 and 3)

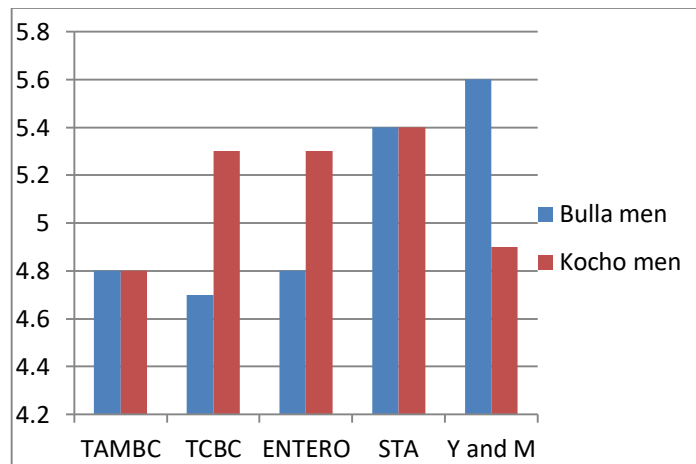


Fig .3. Microbial Counts of Bulla and Kocho (log₁₀ CFU/g)

TAMB: Total Aerobic Mesophilic Bacteria, TCB: Total Coliform, ENTRO: Enterobacteriaceae, STA: *Staphylococci*, YandM: Yeast and Moled

Table 4. Analysis of the cultural and morphological characteristics

characteristic	<i>S.aureus</i>	<i>Salmonella</i>
Gram staining	Positive (purple/violet)	Negative Pink/red
Shape	Spherical(cocci)	Rod-shaped (bacilli)
Catalase test	Positive	Negative
Coagulase test	Positive	Not applicable
KOH test	Not stringiness	Stringy reaction

Prevalence of *S._aureus* and *Salmonella* in kocho and Bulla

The microbiological assessment of kocho and bulla samples showed varying levels of contamination by *S. aureus* and *Salmonella*. Out of 54 kocho samples, 24 (44.4%) tested positive for *S. aureus*, which was higher compared to bulla, where 19 samples (35.1%) were positive. Similarly, *Salmonella* was detected in 4 kocho samples (7.4%) and 3 bulla samples (5.5%), indicating relatively low but notable contamination in both products. Overall, from the 108 samples analyzed, 43 (39.8%) were positive for *S. aureus* and 7 (6.5%) for *Salmonella*. These results suggest that kocho exhibited slightly higher contamination rates than bulla, particularly with *S. aureus*, though both products carried a public health risk due to the presence of pathogenic bacteria. (Table 5),

Table. 5 Prevalence of *S.aureus* and *Salmonella* in Kocho and Bulla

Sample type	Sample size (108)	Number of <i>S.aureus</i> Positives sample (%)	Number of <i>salmonella</i> Positives sample (%)
Kocho	54	24(44.4)	4(7.4)
Bulla	54	19(35.1)	3(5.5)
Total	108	43(39.8)	7(12.9)

Antimicrobial susceptibility pattern of *Salmonella* spp.

The antimicrobial susceptibility results of *Salmonella* isolated from kocho and bulla show a clear pattern of resistance to several commonly used antibiotics. All isolates (100%) were resistant to Ampicillin, indicating that this antibiotic is completely ineffective against the tested strains. Moderate resistance was observed to Ciprofloxacin (42.8%), Gentamicin (57.1%), and Tetracycline (57.1%). Some isolates showed intermediate sensitivity to Ciprofloxacin (28.5%), Gentamicin (14.2%), and Tetracycline (14.2%), while only a few were fully sensitive to these agents (28.5% each) (Table 5). Overall, the findings suggest that *Salmonella* isolates from both traditional fermented products exhibit multidrug resistance, which may pose a potential health risk if these foods are improperly processed or handled. This highlights the importance of hygienic processing practices and prudent antibiotic use to prevent the spread of resistant strains. (Table 6),

Table.6 Antimicrobial susceptibility patterns of *Salmonella* isolated from Kocho and Bulla

Antimicrobial agent	Disc concentration (µg/ml)	Code	Resistance	Intermediate	Sensitive
			Number %	Number %	Number %
Ampicillin	10	AMP	7(100)	-	-
Ciprofloxacin	5	CIP	3(42.8)	2(28.5)	2(28.5)
Gentamicin	10	CN	4 (57.1)	1(14.2)	2(28.5)
Tetracycline	30	TE	4(57.1)	1(14.2)	2(28.5)

Antimicrobial susceptibility patterns *S. aureus* isolation from kocho and bulla

The antimicrobial susceptibility testing of *S. aureus* isolates from kocho and bulla revealed a high level of resistance across all tested antibiotics. All isolates (100%) were resistant to ciprofloxacin, while resistance to gentamicin was 95.34%, with a small proportion (4.65%) showing intermediate susceptibility. Resistance to penicillin G and tetracycline was slightly lower, at 76.74% and 79.06%, respectively, with a few isolates exhibiting intermediate or sensitive profiles (Table 7). These results indicate widespread multi-drug resistance among *S.*

aureus isolates in fermented enset products, emphasizing the need for stringent hygiene practices during processing and careful antibiotic use to mitigate public health risks (Table 7).

Table 7. Antimicrobial susceptibility patterns of *S. aureus* isolated from Kocho and Bulla

Antimicrobial agent	Code	Disc concentration (µg/ml)	Resistance Number %	Intermediate Number %	Sensitive Number %
Ciprofloxacin	CIP	10	43(100)	-	-
Gentamicin	CN	5	41(95.3)	2(4.6)	-
Penicillin G	P	10	33(76.7)	7(16.2)	3(6.9)
Tetracycline	TE	30	34(79)	5(11.6)	4(9.3)

Multiple drug resistance patterns of *S.aureus* and *Salmonella spp*,

The multiple drug resistance (MDR) patterns of *S. aureus* and *Salmonella* isolates from kocho and bulla showed considerable variation. Among 43 *S. aureus* isolates, most were resistant to three or four antibiotics, with 9.3% resisting Gentamicin/Ciprofloxacin/Tetracycline and 4.6% resisting CIP/TE/CN/P. A smaller proportion (2.3% each) resisted only two antibiotics, either CIP/P or TE/CIP (Table 7). In contrast, *Salmonella* isolates displayed higher MDR prevalence relative to their smaller sample size (7 isolates), with 42.8% resisting AMP/CIP/TE, 28.5% resisting CIP/CN/TE, 14.2% resisting AMP/CIP, and 14.2% resisting four drugs (AMP/CIP/CN/TE). These findings indicate the presence of multi-drug resistant pathogens in fermented enset products, underscoring significant public health concerns and the need for improved food safety measures. (Table 8),

Table 8. Multiple drug resistance patterns of *S.aureus* and *Salmonella* spp isolated from Kocho and Bulla.

Isolates		Resisted	Percent %
<i>Staphylococcus</i>	2	CIP/P	1(2.3)
<i>Aureus</i> (43 isolates)	2	TE/ CIP	1(2.3)
	3	CN/ CIP/ TE	4(9.3)
	4	CIP/ TE/ CN/ P	2(4.6)
<i>Salmonella spp</i>	2	AMP/ CIP	1(14.2)
(7 isolate)	3	AMP/ CIP/ TE	3(42.8)
	3	CIP /CN/ TE	2(28.5)
	4	AMP/ CIP/ CN/ TE	1(14.2)

AMP-Ampicillin CIP- Ciprofloxacin CN- Gentamicin P- Penicillin TE- Tetracycline

5. DISCUSSION

The findings of this study reveal that all respondents reside within the Dega agro-ecological zone, where enset serves as the primary staple crop and a vital pillar of household food security. The dominance of enset cultivation for food purposes, along with its additional uses for income generation and traditional medicine, underscores its multifaceted cultural and economic significance in the community. Most participants perceived enset-based foods as superior in both taste and nutritional quality compared to other crops, reflecting deep-rooted culinary preference and nutritional awareness. The universal agreement regarding the adequacy of family labor and the safety of enset consumption indicates a strong foundation of indigenous knowledge and practical experience related to its production and use. Although kocho and bulla were consumed by nearly all households, variations in their frequency of consumption point to possible differences in household preferences, accessibility, or seasonal availability.

This study assessed the microbial quality and safety of kocho and bulla, two traditional enset-based food products, and revealed notable variations in their microbial profiles. These differences may reflect variations in processing methods, handling practices, or storage conditions among households, which in turn influence the overall safety and quality of the products. Kocho was found to have higher counts of total aerobic mesophilic bacteria (TAMB), total coliform bacteria (TCCB), Enterobacteriaceae, and *Staphylococci*, whereas bulla showed lower bacterial loads but higher yeast and mold counts. The high bacterial load in kocho is likely linked to its traditional preparation and prolonged fermentation in underground pits, which create favorable conditions for microbial growth. Similar findings were reported in southern Ethiopia, where kocho samples were shown to harbor high coliform and aerobic mesophilic counts due to unhygienic processing and storage (Awulachew, 2021).

The elevated coliform levels observed in this study also agree with earlier Ethiopian reports that associated kocho with fecal contamination resulting from the use of unclean water and utensils (Awulachew, 2021). bulla, in contrast, exhibited significantly lower bacterial contamination. The mean total coliform count was higher in kocho (5.3 log CFU/g) than in bulla (4.7 log CFU/g), indicating a greater level of coliform contamination in Kocho, Enterobacteriaceae counts were elevated in kocho (5.3 log CFU/g) compared to bulla (4.8 log CFU/g), but higher yeast and mold counts were greater in bulla (5.6 log CFU/g) than in kocho (4.9 log CFU/g). This pattern may be

due to its shorter processing period and higher moisture content, which discourage bacterial growth while promoting fungal proliferation. Comparable observations were made in Sidama and Gurage zones, where bulla samples recorded lower bacterial loads but were vulnerable to fungal contamination during drying and storage mold (Zorina & Obozova, 2011). However, unlike some Ethiopian studies that documented relatively low mold counts in bulla, the present findings revealed higher fungal loads, suggesting differences in processing or environmental conditions (Galmessa *et al.*, 2005).

The detection of Enterobacteriaceae and *Staphylococci* in both kocho and bulla points to hygiene-related issues. *Staphylococcus* species are often introduced through human handling, as they are part of the natural flora of the skin and nasal passages. This agrees with previous Ethiopian studies on fermented foods, which identified human handling as a major source of *Staphylococcal* contamination (Zorina & Obozova, 2011) Such contamination raises food safety concerns, particularly when the products are consumed without sufficient cooking.

The results revealed higher contamination of *S. aureus* in kocho (44.4%) than in bulla (35.1%), while *Salmonella* occurrence was relatively low in both products. The higher prevalence of *S. aureus* in kocho may be associated with extended fermentation, handling, or storage conditions that favor its growth, as reported by (Kebede *et al.*, 2021). Although *Salmonella* levels were low, its detection still indicates possible hygiene lapses during processing or post-fermentation storage, consistent with the findings of (Tamang *et al.*, 2020).

Overall, both products present a moderate public health concern, emphasizing the need for improved sanitation and hygienic handling during fermentation and storage (WHO, 2020). Both *S. aureus* and *Salmonella* isolates from kocho and bulla exhibited multiple drug resistance, with *S. aureus* showing resistance mainly to three or four antibiotics and *Salmonella* displaying even higher MDR rates despite fewer isolates. Similar trends were reported by Tadesse,(2020), who found high levels of antibiotic-resistant foodborne pathogens in traditional fermented foods in Ethiopia. Resistance to commonly used antibiotics such as ciprofloxacin, tetracycline, and ampicillin may result from the frequent or improper use of antimicrobials in humans and livestock, as noted by (Gislin *et al.*, 2018). The detection of MDR pathogens in fermented enset

products poses a potential public health risk, emphasizing the need for strict hygiene practices and antimicrobial resistance monitoring during production and storage (WHO, 2020).

6. CONCLUSION and RECOMENDEATION

6.1 CONCLUSION

The study demonstrated that enset is the primary staple crop in the Dega agro-ecological zone, with most households producing it for food and recognizing its role in year-round food security and income generation. kocho and bulla are widely consumed and culturally significant, with family labor sufficient for their production. Microbial analysis indicated that kocho tends to have higher bacterial loads, while bulla shows greater fungal presence; however, both products were generally considered safe for consumption. These findings highlight the importance of maintaining hygiene during processing, optimizing fermentation practices, and ensuring proper storage to reduce microbial contamination. Promoting producer education and monitoring food safety can enhance the quality and safety of kocho and bulla, supporting both household nutrition and cultural practices. The microbiological assessment revealed notable contamination levels of *Staphylococcus aureus* and *Salmonella* in kocho and bulla, with kocho exhibiting higher rates of contamination

6.1 RECOMENDEATION

This study has limitations, including reliance on conventional microbiological methods and sampling restricted to a single district, which may not capture seasonal or regional variations. Future research should investigate the effect of improved fermentation pits, controlled drying techniques, and the use of starter cultures on reducing microbial load in kocho and bulla.

kocho and bulla fermentation process are very important to improve, standardize and modernized traditional Enset processing systems and help to minimize time and energy needed, enhance quality and quantity of food product and also minimize public health problems.

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9. APPENDIX

9.1 APPENDIX



Fig.1 Kocho bulla and fermentation and sit along pared of time method



Fig.2 Laboratory equipment

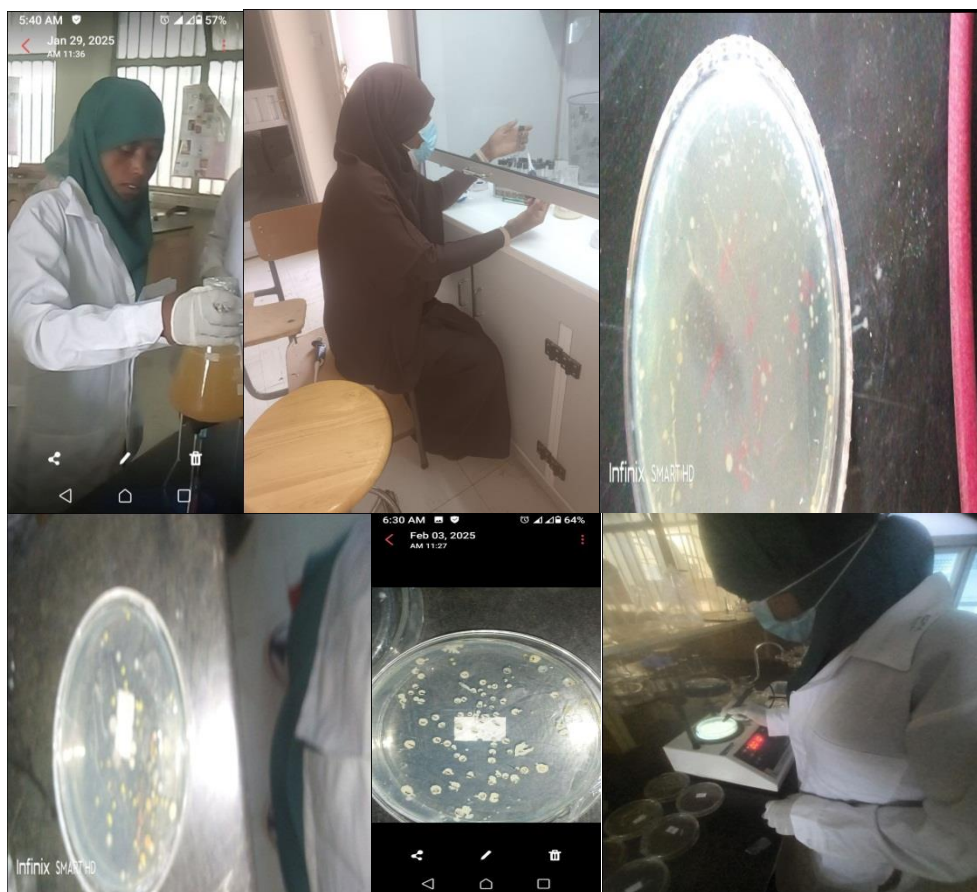


Fig.3 serial dilution and pouring media on Nutrient agar media and colony count
Example. TAMC and TCC



Fig.4 Fungal media preparation on PDA and colon count Yeast and moled



Fig.5 Media preparation on XLD agar and colony counting *salmonella*

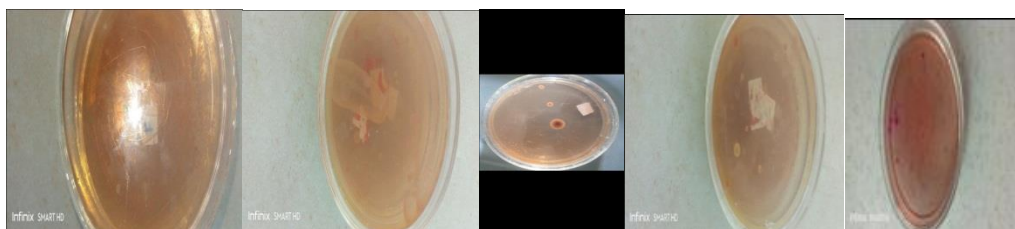


Fig.6 Media preparation on MacConkey agar and colony counting *Entrobacterace*



Fig. 7 Catalase taste identifying gram positive and gram negative



Fig. 8 (KOH) tastes of salmonella



Fig.9 gram staining and microscopic observations

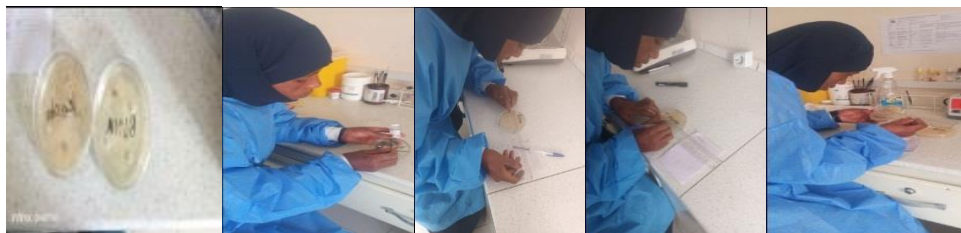


Fig.10Antimicrobial susceptibility testing of isolated bacteria antibiotic test and measuring

9.3 APPENDIX

Household survey questionnaire

Date_____Family size_____gender ender: Male _____ Female_____

Marital status: Married_____ Unmarried_____ Widowed_____ Divorce_____

Education status: _____occupation:_____

Region_____Zone_____Woreda_____Kebele_____

1. What is your main goal for producing Enset?

A. Food B. Income in cash C. Medicine D. Other (details)

2. Do you think that Enset is more crucial than other crops to ensuring a year-round supply of food? A. Yes B. No

3. Do you believe that Enset is more crucial to producing cash income than other crops? A. yes
b. no

4. Do you think Enset food is better than other food types? A. Yes B. No

5. How long does it take for the kocho to ferment and become fit for eating? A. Less than two weeks B. Two to four weeks C. one to two months D. More than two months

6. Do you keep Kocho in storage? A. Yes B. No

7. If yes, which storage technique do you use?"

A. Underground storage (pit storage) B. Wrapping with Enset leaves and mid ribs and storing within home C. Other (specify)

8. How long is fermented kocho kept in storage?

A. Less than one month B. 1 to 2 months C. 2 to 6 months D. 7 to 12 months E. More than one yea

9. Kocho management during storage

Activity	Time interval
Changing wrapping leaves	
Changing storage pit	

10. Do you believe that microbes exist in Kocho and Bulla? A. Yes B. No

11. If the answers to the previous questions are yes, when are the microbial load group's present in Kocho?

A. Following fermentation; B. Prior to fermentation; and C. During fermentation

12. Is the labor provided by your family sufficient for the production of kocho and bulla?

A. In agreement; B. in disagreement

13. Does your family eat Kocho & Bulla? A. Yes B. No

14. Do you have any experience using Bulla and Kocho products? A yes B.No

15. Do you think there is problem with consumption of Kocho and Bulla product?

A. Yes B. No

16. Do you always use Bulla and Kocho products? A. Yes B. No

9.4 APPENDIX

Ethical clearance

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The Federal Democratic Republic of Ethiopia
Ministry of Education
Werabe University
Academic Vice President
Ref.No: 06/1261/251/16
Date: 06/12/2016

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ከላይ በርዕስ ለመጥቀስ እንደተሞከረው ከዚህ በታች ስማቸው የተዘረዘሩ ተማሪዎች በወራቤ ዩኒቨርሲቲ በተፈጥሮና ቀመር ሳይንስ ኮሌጅ በባዮሎጂ ትምህርት ክፍል በማይክሮ ባዮሎጂ የትምህርት መስክ የቦለትና ድግሪያቸውን በመማር ላይ የሚገኙ ሲሆኑ ከመመረቁያ ጽባቶቻቸው ጋር በተያያዘ የMicrobial and Bacteriological Analysis ትምህርት በወራቤ ኮ/ሰ/ሆስፒታል በሚገኘው ቤተ መከራ/ላብራቶሪ/ ለመስራት ስለፈለጉ ተማሪዎች ለሰራ በሚመጡበት ወቅት በናንተ በኩል ትብብር እንደደረግላቸው እክብሮት እንጠይቃለን።

S/N	Students Name	Selected titles
1	Keyre Sirgota	Microbiological Quality and Safety of Common Street Vended Foods Sold in Werabe Town, Silte Zone, Central Ethiopia Region, Ethiopia
2	Muhammed Mustefa	Isolation and Characterization of Amylase Producing Microorganisms from Soil Samples around Werabe Town, Silte Zone, Central Ethiopia Region, Ethiopia
3	Yasin Getiso	Physico-chemical and Microbial Quality of Raw Cow Milk Collected from Dairy Farmers and marketed in kibat administrative town Silte Zone, Central Ethiopia Region, Ethiopia
4	Alima Halil	Microbiological Quality and Safety of Kocho and Bulla Collected from Alichu Wuriro Woreda, Silte Zone, Central Ethiopia Region, Ethiopia
5	Mekya Ebrahim	Physico-chemical and Bacteriological Quality of Drinking Water of Different Sources in Werabe Town, Silte Zone, Central Ethiopia Region, Ethiopia

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