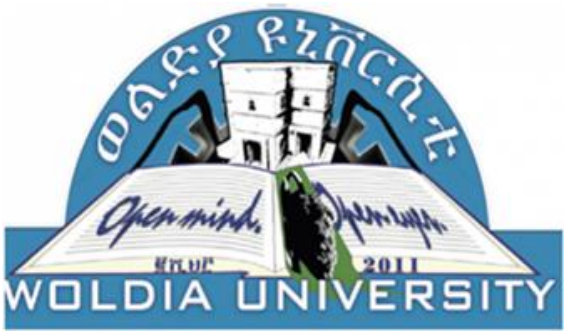

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF BIOLOGY

ISOLATION AND CHARACTERIZATION OF DOMINANT YEAST SPECIES FROM TELLA AND TEJ IN RAYA KOBO, NORTH WOLLO, ETHIOPIA

BY:

DESYE ZELELE FENTAW

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF WOLDIA UNIVERSITY IN PARTIAL FULFILLMENT FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN APPLIED MICROBIOLOGY

MARCH, 2025

WOLDIA,ETHIOPIA

Isolation and Characterization of Dominant Yeast Species from Tella and Tej in Raya kobo, North Wollo, Ethiopia

A Thesis Submitted to the Department of Biology
School of Graduate Studies
Woldia University

In Partial Fulfillment of the Requirements for the Degree of
Master of Science in Applied Microbiology

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March, 2025
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Approval Sheet

As thesisresearch advisor, we hereby certify that, we have read and evaluated this thesis prepared, under our guidance byDesye Zelele Fentaw entitled "Isolation and Characterization of Dominant Yeast Species from Tella and Tej in Raya Kobo, North Wollo, Ethiopia", and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Applied Microbiology compiled with the regulations of the university and needs the accepted standards with respect to originality and quality. We recommend that it can be submitted as fulfilling the thesisrequirement.

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Co Advisor Signature Date

As member of the Board of Examiners of the Master of Sciences (MSc.) thesis open defense examination, we have read and evaluated this thesis by Desye Zelele entitled “Isolation and characterization of dominant yeast from Tella and Tej in Raya Kobo, North Wollo, Ethiopia”We hereby certify that; the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (MSc.) in Applied Microbiology.

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Name of Internal Examiner Signature Date

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Name of External Examiner Signature Date

DEDICATION

This paper is dedicated to my family, whose steadfast support and encouragement have been my unwavering source of strength throughout this journey. Their belief in my abilities and their constant motivation have inspired me to pursue my goals with determination and passion.

To my adivisors I express my heartfelt gratitude for your invaluable guidance, wisdom, and constructive feedback. Your insights and encouragement have not only enriched my work but also deepened my understanding of the subject matter. I am truly grateful for the collaborative spirit we shared, which has elevated this research.

Finally, I hope that this work resonates with all those who seek knowledge and understanding. May it foster a spirit of exploration and discovery, inspiring others to delve deeper into the complexities of our world and strive for continuous learning.

AUTHOR DECLARATION

By my signature below, Ideclare and affirm that thatthismasterthesisEntitled“Isolation and characterization of dominant yeast from Tella and Tej in Raya Kobo, North Wollo, Ethiopia” is my original work. I also seriously declare that this thesis has not beensubmitted to any other institution any where fortheawardofanyacademicdegree,diplomaorcertificate.Allsources of materials that are used for this thesis have been duly acknowledged throughcitation..

Name: Desye Zelele

Signature _____

Date _____

BIOGRAPHICAL SKETCH

The author, Desye Zelele, was born on June 13, 1987, in Raya Kobo, Ethiopia. He attended elementary and secondary school in Raya Kobo Woreda, at Kalim Elementary School and Woldia General Secondary School, respectively. After successfully passing the Ethiopian Higher Education Entrance Qualification Certificate (EHEEQC), he earned his Bachelor of Science degree in Applied Biology from Samara University in July 2011. Following his graduation, he served as a marketing officer at the Raya Kobo Trade and Market Office until he joined Woldia University for his postgraduate studies.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my advisor, Dr. Sultan Mohammed, and my co-advisor, Dr. Esubalew Sinte, for their invaluable guidance, encouragement, and critical feedback throughout the course of this study. I am also grateful to my colleagues for their support and encouragement. Special thanks go to my family for their financial and emotional support, which enabled me to complete this research

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ABBREVIATIONS AND ACRONYMS

CFU	Colony Forming Unit
CSA	Central Statistical agency of Ethiopia
EC	Electric Conductivity
FAO	Food and Agriculture Organization
Rpm	Revolution per minute
SCP	Single Cell Protein
TDS	Total dissolved substance
Temp	Tempreture
WHO	World Health Organization
WUJY	Woldia University Tej Yeast
WUTY	Woldia University Tella Yeast
YPD	Yeast Peptone Dextrose

ABSTRACT

Ethiopia is renowned for its rich cultural heritage, which includes the production and consumption of traditional alcoholic beverages, particularly Tej and Tella A cross-sectional study was conducted in the Raya Kobo district, northeastern part of Ethiopia between February 2023 and March 2024, focusing on the isolation and characterization of yeast species from traditionally prepared Tella and Tej. A total of thirty (30) vending houses from Kobo and Robit towns were included in the study, allowing for a comprehensive sampling of these popular local beverages.The result reveiled that, the yeast species isolated from Tella included Saccharomyces cerevisiae (5.2×10⁶CFU/mL), Zygosaccharomyces bailii (4×10⁴ CFU/mL), Hanseniaspora species *(3×10⁴ CFU/mL), and Pichia pastoris (3×10⁴CFU/mL). Similarly,Tej samples contained Saccharomyces cerevisiae (4×10^{6}CFU/mL), Zygosaccharomyces bailii (3 × 10⁴ CFU/mL), Hanseniaspora* species *(2×10⁴ CFU/mL), and Pichia pastoris (3×10⁴ CFU/mL).The results revealed that Saccharomyces cerevisiae was the predominant yeast species in both Tella and Tej. Specifically, These findings highlight the role of yeast primary fermenting agent responsible for the flavor, aroma, and alcoholic content of these traditional drinks.*

***Keywords*: Isolates, Raya Kobo, Saccharomyces cerevisiae,Tej, Tella.**

1. INTRODUCTION

1.1. Back Ground of The Study

In nearly all areas of the world, some types of alcoholic beverage native to their region are prepared and consumed (Buglass 2011). In Ethiopia, some of the fermented beverages include Tella, Tej, Borde and Shamita which are very popular traditional drinks. The latter two, Borde and Shamita are mainly prepared in central and southern Ethiopia (Lee, Regu *et al*. 2015). By considering popularity, traditional alcoholic beverages namely, Tella and Tej are very common in northern part of Ethiopia. Cereals, legumes and tuber roots are the major raw materials which are mostly fermented but milk, fish and meat are also used (Akabanda *et al.*, 2014). Depending to the country or even the locality, various names may be given to the same products that are basically similar with slight variations on production and raw materials. Yeasts are suitable for modern biotechnology applications for various reasons. For example, yeasts offer the ease of microbial growth and gene manipulation allowed to understand many eukaryote-specific post-translational modifications, such as proteolytic processing, folding, disulfide bridge formation, and glycosylation *(*Jans *et al.*, 2017).Also, modern biotechnology applications of yeasts involve foreign proteins expressing for the pharmaceutical industry, the production of biofuel, organic acids, bioethanol, industrial enzymes, and small molecular weight metabolites (Türker, 2014).

More recently, yeasts species including *Pichia pastoris,Saccharomyces cerevisiae* and others have been developed for heterologous proteins and enzymes production including pharmaceutical proteins, and as for the delineation of genes and their function in mammalian and human metabolism and disease processes (Cyert and Philpott, 2013). Yeasts under species of *Saccharomyces* were present in Tella at the middle of fermentation however reduced at last and also presence in the raw material were confirmed by Berhanu, (2014). According to (Getachew Tafere, 2015), *Saccharomyces* spp. and *Lactobacillus* spp. were among the fermenting organisms. A study conducted to explore the micro biota of Tej by Mulaw and Tesfaye, (2017), the dominant yeast, *S. Cerevisiae*, counts ranged from 10^2 to10^3cfu/g in gesho samples while 10^1 to 10^2cfu/g in honey samples. Attempts had been made on studying stress tolerance on Tella yeast on their ability to survive in high ethanol concentration, sugar, through checking their viability (Andualem *et al*., 2017).

Saccharomyces cerevisiae is the most domesticated yeast and still widely industrial today. This microorganism has become a valuable tool to study eukaryotic cells because it harbors many enzymes and pathways with counterparts in mammalian cells (Tomo *et al*., 2013).

Several researchers have studied yeasts in food industries, in different medical agricultural and environmental applications worldwide (Willaert, 2017). Many genera or species of yeasts have been reported in relation to various biotechnology applications and the usage of molecular tools in recent years has helped to clarify the nomenclature confusion. Traditional fermented foods from Africa remain the main source of nutrition for many rural communities. They serve as main course meals and beverages or as food condiments.

In the first case they are made from carbohydrate rich raw material, while the latter are usually made from seeds rich in proteins. Cereals (maize, rice, sorghum and millet), legumes and tuber roots are the major raw materials used in Africa, but also milk, fish and meat are fermented (Muyonga, Nansereko *et al*. 2020). Ethiopia is one of the countries where a wide variety of traditional fermented beverages are prepared and consumed. The various traditional fermented beverages consumed in Ethiopia consist of both high alcoholic and low alcoholic beers.

The traditional beverages deal with popular products such as Kribo, Borde, Areki, Tej and Tella. The difference in nutritional content pH and other chemical properties of the products are presented traditional in relation to household processing. According to Mogessie (2006*)*, microbiological study on Ethiopian traditional fermented foods and beverages has started in the 1980s on topics like microbial succession and accompanying changes, food safety, processing and as well as food spoilage.

Alcohol in traditional beverages serves as source of calories valuable to the calorie-deficient villager. Ethanol has gross calorific values of 30 MJ/Kg (Getaye, Tesfaye *et al*. 2018). The primitive beverages provide not only calories but also vitamin B and proteins due to residues of the substrates, the fermenting yeasts and other microorganisms (Kaur, Ghoshal *et al*. 2019).

1.2. Statement of the Problem

The traditionally fermented beverages are low-cost product in all aspect as they are usually manufactured using only rudimentary equipment. Because of their cheapness, low-income

groups mostly consume them. Thus, their handling and consumption often takes place under conditions of poor hygiene. Most of the customs and rituals involving the Ethiopian traditional fermented beverages are still prevailing, today in urban areas, village communities and rural households. Fermentation of Tejand Tella like other traditionally fermented alcoholic beverages relies on the microorganisms present in the substrates, fermentation vats, or equipment's (Abebe 2011). In Ethiopia, traditional alcoholic beverages such as Tella and Tej play a significant cultural and social role.

These beverages are commonly prepared and consumed in local communities, often using traditional methods that vary from region to region.

However, the microbial composition of these beverages, particularly the yeast species involved in their fermentation, remains poorly understood(Minbal 2021). Yeast species play a crucial role in the fermentation process, influencing the quality, flavor, and safety of the final product. While *Saccharomyces cerevisiae* is known to be a dominant yeast in alcoholic fermentation, little research has been conducted to identify and characterize the dominant yeast species present in traditionally brewed Tella and Tej(Vilela 2019). Moreover, the lack of standardization in preparation and handling of these beverages raises concerns about potential microbial contamination and its impact on public health. As these beverages gain popularity beyond local consumption, there is a pressing need for scientific investigation into their microbiological profiles(Voidarou, Antoniadou *et al*. 2020).

The isolation and characterization of the dominant yeast species from these beverages are essential not only for ensuring their quality and safety but also for understanding the microbial diversity in traditional Ethiopian fermentation practices. This study aims to fill this gap by isolating and characterizing the yeast species present in Tella and Tej, providing a foundation for improving fermentation practices and ensuring the global marketability of these beverages.Thus study aimed to investigate the Isolation And Characterization Of Dominant Yeast Species From Tella And Tej In Raya Kobo, North Wollo, Ethiopia.

1.3. Significance of the Study

This study would provide information about potential yeast isolates found in both Tella and Tej. In addtion to make awareness on the dominant yeast in traditional alcoholic

beverage of Tella and Tej for the peoples living in the place of Raya kobo. This study may provide significant information for related works.

1.4 Scope of the Study

The scope of this study was delimited only toisolation and characterization of the dominant yeast fromTella and Tej at Raya kobo District Ethiopia.

1.5 Research Question

2. What is the level of knowledge and what practices do vendors follow regarding the fermentation process of Tella and Tej?
3. How knowledgeable are vendors about the preparation methods of Tella and Tej?
4. Which dominant yeast species can be isolated and characterized from traditional alcoholic beverages, Tella and Tej?
5. What are the colony-forming units (CFU/mL) of yeast species found in samples of Tella and Tej?

1.6. Objectivesof the Study

1.6.1 General objective

The general objective of the study was to isolate and characterize the dominant Yeast species from Tella and Tej in Raya Kobo District, Ethiopia.

1.6.2 Specific objectives

The specific objectives of the study were to:

- Assess the knowledge and practices of vendors regarding the fermentation process of Tella and Tej.
- Evaluate the Knowledge of Vendors on Tella and Tej Preparation
- Isolate and characterize the dominant yeast species present in traditional alcoholic beverages, Tella and Tej.
- Enumerate the colony-forming units (CFU/mL) of yeast species in both Tella and Tej samples.

2. LITERATURE REVIEW

2.1. Ethiopian Traditional Alcoholic Beverages

Ethiopia is one of the countries where a variety of traditional fermented beverages are prepared and consumed. The various traditional fermented beverages consumed in Ethiopia consist of high alcoholic beers such as Tella; low alcoholic beers such as keribo, buqri, shameta, borde, and wine such as Tej made from honey and distilled spirits such as katikala or areki (Getaye, Tesfaye *et al*. 2018). The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. Yeast plays a vital role in the production of all alcoholic beverages and the selection of suitable yeast strains is essential not only to maximize alcohol yield, but also to maintain beverage sensory quality.Yeasts are involved in the production of Africa traditional fermented aklcoholic beverages. In several types of alcoholic beverage, yeasts are main microorganisms isolated and *Saccaromyce cerevisiae* was the main species (Mogmenga *et al.*, 2017).

The yeast species that dominates in the production of alcoholic beverages worldwide is *Saccharomyces cerevisiae*, and the particular strains of this species employed in fermentation exert a profound influence on the flavoure and aroma characteristics of different beverages. For large-scale beverage fermentations, as in brewing, wine making and distilled spirit production, pure cultures of selected strains of *S. cerevisiae* are usually used. These strains are either sourced in house or supplied from yeast producing companies. In smaller-scale (artisanal) processes, spontaneous fermentations may be allowed to occur that rely on indigenous microbiological flora (wild yeasts and bacteria) present in the raw material and in the production facility. in some types of alcoholic beverage fermentations, non-*S. Cerevisiae* yeasts may be employed either as starter cultures, or occur naturally. For example, in wine making the *S.cerevisiae* yeast strain used (Walker and Stewart 2016).

2.1.1. Tella

Tella has various vernaculars' in the various regions and is a malt beverage based on substrate such as *barley, wheat, maize, millet, sorghum, teff or other cereals. It is by far, the most commonly consumed alcoholic beverage in Ethiopia.* During fermentation, the change of temperature is inevitable and can be originated from the climate change and biochemical reaction along de polymerization carbohydrate (Arroyo-Lopez *et al.,* 2009).

Temperature is a very useful factor on the alcoholic beverage fermentation and which has been previously showed the influence on growth rate of *S. cerevisiae* with positive and linear effect in the interval 15-35ºC (Arroyo-Lopez *et al.,* 2009). *Tella* is brewed from barely (Hordeum vulgare), wheat (*Triticum aestivum),* maize (*Zea mays*), millet (*Eleusine coracana*), sorghum (*Sorghum bicolor), tef* (*Eragrostis tef),* gesho (*Rhamnus prinoides*) and variety of spices (Wilson 2021). Grains mainly provide starch, which is made up of long chains of glucose units, and production of ethanol is necessarily breaking down the chains of this carbohydrate for obtaining glucose syrup (Vohra *et al*., 2014).

Although the way of preparing *Tella* differs among ethnic groups, traditions, and economic situation, the basic processing steps are similar (Getachew, 2015). A container, which is used for *tella* preparation, is usually cleaned with water and leaves of grawa (*Vernonia amygdalina*) and then fumigated by the smoke of burning chips of weyra (*Olea europaea)* to eliminate some adverse microorganisms and to get the desirable flavor of the fermented product (Getaye *et al*., 2018). Leaves and stems of gesho plant (*Rhamnus prinoides*) are chopped, sun-dried and pounded into finer powder to make beverages biologically stable and impart the typical bitter taste (Adugna, Mohammed et al. 2020). Malt (*bikil)* is a major source of the dominant yeast in tella and can be prepared from different grains: barely, wheat, maize, and sorghum or finger millet. The grains are first cleansed of all chaff, broken kernels and extraneous materials, rinsed repeatedly followed by soaking in water for one day. After draining excess water, the grains are wrapped in false banana (*Ensete ventricosum*) or other large leaves and kept moist. The germination takes 2 days for finger millet and 3 days for other cereals. After removal of non-germinated grains, germination is halted by sun drying.

The malt is then stored separately in a dry place until required (Adugna, Mohammed *et al.* 2020). Flour of millet or barely or Tef(dark variety) is toasted, milled, mixed with water and baked on a wide metal pan into *kita* (a thin, 5 to 10 mm thick, pancake- like bread). Barely flour is toasted on *mitad*, sprinkled with water and toasted until it becomes dark brown to form what is known as *enkuro* (Getaye *et al*., 2018).

Kita (unleavened bread) and enduro (toasted flour) are determinant of tella flavor and color which may vary from dark brown to dark (Chala, 2019). When all the necessary ingredients are ready, Tella fermentation process has different phases and is then provided for consumption either filtered to yield what is popularly known as filter-tella or diluted with water and drank as a regular Tella (unfiltered Tella).

Several studies analyzed quality of Tella with respect to Ethanol level, pH, specific gravity and color, and range of results are 2-8% (v/v), 4-5, 1.003-1.008 and 40-70, respectively (Getachew, 2015). Very common container for preparation of tella has been clay pot (insira or gan) (Getachew, 2015). However, nowadays people are using plastic container for preparation. In fact, plastic containers are affordable and non-fragile as compared to clay pot. In Ethiopia, people claim that the quality of tella prepared using clay container and plastic jar has significant difference. Amhara Tella has Gesho (*Rhamnus prinoides*) and is more concentrated. Gurage Tella is delicately aromatized with a variety of spices. Oromo Tella has no Gesho (*Rhamnus prinoides),* and it is thick and sweet (MARU 2021). There are 3 types of Tella generally Zillil, Corefe and lifter all contain hops. Tella is made from different cereals. Teff and corn are the most popular, but in some areas barely, millet or sorghum can be used. The way of preparing Tella differs among the ethnic groups and depends on traditional and the economic situation (Lee, Regu *et al*. 2015).

The clay container (insera) and bermel are washed with Grawa (*Vernonia amygdalina*) and water several times and after that smoked with 5wood from Weira (*Olea europaea subsp. Cuspidate*) for about ten minutes, in order to get it as clean as possible. Germinated grain of barley, or corn, or wheat (bikil), bought in the local market or prepared at home, are dried and milled. For making bikil, the grains are moistened in water and the moist grains are placed between fresh leaves, left to germinate for 3 days and after that dried. Gesho *(Rhamnus prinoides*), local hops, is available dried in the local market (Mulaw and Tesfaye 2017).

The leaves of gesho (*Rhamnus prinoides*) is dried again in the sun for about ½ hour and after that pounded. The leaves are separated from the stems, which need a longer time to dry. The ground gesho *(Rhamnus prinoides*) leaves are placed in a clay container with water and left to ferment for 2-3 days. Gesho (*Rhamnus prinoides*) is responsible for the bitter taste of tella and the bitterness of tella is directly related to the amount of gesho added during brewing.

It is also thought to be the source of various chemicals (Zewdu, Tsehai *et al*. 2023). It is assumed that gesho (*Rhamnus prinoides)* maintains acidic pH during tella fermentation so as to modify the nature of the mash or difdif and inhibits the growth of undesirable microorganism.

Some of the grains intended for Tella preparation are toasted and milled, and then mixed with water and baked on the mitad to prepare what is known as kita or meketa (a thin, 5-10 mm thick, pancake-like bread). This *tella kita* , *abshilo* or *meketa* at Raya kobo, broken into small pieces, part of the milled bikil and the pounded gesho stems are added to the water and allowed to ferment for 1-2 days. The rest of the flour is toasted on mitad, sprinkled with water and toasted until dark brown to form what is known as enkuro. This mixture enkuro, the rest of the germinated grains (bikil), some gesho (*Rhamnus prinoides*), and water are added to the container. The mixture is kept covered overnight, after which more water is added and the container is kept sealed for 5-7 days, until when the beverage is ready. Tella can be kept for 10-12 days (Ashenafi 2006).

High quality (concentrated) Tella is made with a relatively small quantity of water (Debebe, 2006). There are several recipes for making Tella and it appears as if every housewife has her own version of the ingredients required. The biochemical changes, the microorganisms involved in the fermentation and those which bring about desirable and undesirable changes in the process of Tella making are described. According to the report, the fermentation process of Tella is divided into four phases. The first occurs in the original mixtures of ingredients, and the second and third phases occur after successive additions of more carbohydrate materials (MINBALE 2021).The three main carbohydrate materials are mentioned to be bikil, kitta and enkuro. The latter phase is where acidification takes place, which is actually not desirable.

Maximum ethanol production occurs during the third phase and at the beginning of the fourth phase.

Tella is actually a beverage of variable viscosity and having a variety of colors (ranging from grayish-white to dark brown).Were analyzed for their ethanol, methanol, and fuel oil contents by(Minibal 2021).

2.1.2. Tej

Tej is a home-processed and is often served in a flask- like pitcher or bottle, called a berelle but also commercially available honey wine. It is a beverage mainly used for great feasts, such as weddings and the breaking of fasting. It is prepared from honey, water and leaves of *Gesho(Rhamnus prinoides*). *Tej* is the typical Ethiopian honey-wine or mead. In popular bars it was, and often still is, served in longneck berelle, old perfume bottles, a custom dating from the late 19th century. Sometimes, widely for commercial purposes, mixture of honey and sugar could be used for its preparation. In cases where sugar is used as part of the substrate, natural food coloring is added so that the beverage attains a yellow color similar to that made from honey. Some also add different concoctions such as barks or roots of some plants or secrete herbal ingredients to improve flavor or potency and to attract customers. Due to concoction or combining, adulteration practices and possibly some other reasons, producers usually are not willing to tell about additives used and their composition *(*Bahiru, 2000).

During the preparation of tej, the fermentation pot is seasoned by smoking over smoldering *Rhamnus prinoides* stems and olive wood. One part of honey mixed with 2 to 5% (v/v) parts of water is placed in the pot, covered with a cloth for 2 to 3 days to ferment after which wax and top scum is removed. Some portion of the must is boiled with washed and peeled *Rhamnus prinoides* and put back to the fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15-20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *Rhamnus prinoides (Hotessa and Robe 2020).*

Good quality *Tej* is yellow, sweet, effervescent and cloudy due to the content of yeasts. The flavor of *Tej* depends upon the part of the country where the bees have collected the nectar and the climate (Vogel and Gobezie, 1983). The range for alcohol content of Tej is very wide and values lower than 5% could be obtained in cases where the fermentation is far from complete. The higher titratable acidity combined with significant amount of alcohol would result in sweet-sour alcoholic flavor which would make tej preferable by consumers. This combination would also give Tej the required microbiological stability, which would permit certain conservation without the use for highly specific techniques as observed in wine.

Producers do not determine the end-point of the fermentation and tej is consumed while in the state of active fermentation(Bahiru, Mehari *et al*. 2001).

Aerobic conditions would result in the formation of acetic acid from alcohol making the product sourer, a condition termed as 'dryness' by consumers (Bahiru *et al.,* 2001). Fermentation of tej, like other traditionally fermented alcoholic beverages, relies on the microorganisms present in the substrates, fermentation vats or equipment. As these fermentations are natural and, thus, uncontrolled, alcohol and fusel oils produced during the fermentation can be hazardous to health if produced beyond acceptable levels. With the variable micro flora of such spontaneous fermentation, variability of the product is inevitable (Bahru *et al.,* 2001).

According to Bahru *et al* (2001), *Tej* has fewer carbohydrates than European honey wine and more proteins than grape wine. The regulation of the proportion of honey to water, the dosage of aromatic herbs (gesho, *lat. Rhamnus prinoides; taddwo, lat. Rhamnus staddo)* and the preparation and fermentation time define its alcoholic, gustative and social values, differentiating between good and domestic and commercial, from white to red to black to a bitter one, din, the cheapest and most unpleasant.

2.2 Cleaning Agents of Tella and Tej

2.2.1. Grawa (Vernonia amygdalina Del.)

Grawa popularly known as bitter leaf belongs to the Compositae family. It is widely distributed in most African countries and utilized for various purposes. Different parts of the plant are consumed as a raw vegetable after abating bitterness by boiling and soaking in several water changes.

V. amaygdalina is a good scope to commercialize health supplement for health promoting effect and also as medicinal plant. In some p arts of Africa grawa is regarded as a medicinal plant in Africa to treat fever, kidney problems and stomach discomfort.

Additional studies also showed strong parasiticidal properties (Ogboli *et al.,* 2000) bactericidal effect (Erasto *et al.,* 2006), antifungal property (Iwalokun *et al.,* 2006). In Ethiopia grawa is usually used as ethno medicinal plant for stomach disorders and as a cleansing agent for containers of Tella and Tej fermentation.

Fermentation containers are washed repeatedly using grawa leaves and water till foams appear. The effect of grawa leaves in relation to Tella preparation is not studied yet. However, the leaves are reputed to contain detergent agents (Maru 2021).

2.2.2. Weira (Olea europaea L. subsp cuspidate)

Weira is a sub-species of olive. It is distributed throughout Africa, India and China (Forest *et al.,* 2003). The fruits, leaves and stem of weira consist of series of compounds that

represent multi chemical mechanism of defense against microbe and insect attack (Kubo *et al.*, 1985).

Weira is commonly used in Ethiopia for smoking fermentation containers of traditional beverages such as Tella, and Tej, containers of water, containers of milk and milk products. The smoke is reported to remove and to cause sluggish growth of coli forms in smoked milk and its products (Ashenafi, 2002). At the same time it is also observed to delay milk spoilage. Weira smoke give smoky flavor to water, Tella, Tej and milk. It has been shown that weira smoke contains compounds which have the ability to kill or delay the rate of growth of a number of human pathogenic bacteria and fungi (Ashenafi, 2002).

2.3. Some Important Ingredients of Tella and Tej

2.3.1. Gesho, Rhamnus prinoidesL. Herit Gesho

Gesho is commonly used for preparation of traditional alcoholic beverages, Tella and Tej in Ethiopia. *Rhamnus prinoides* is commonly found in eastern, central and western Africa. It is found in different p arts of Ethiopia, both in the wild and as a cultivated plant. It grows at elevations of 1,400 to 3,200m above sea level. *Rhamnus prinoides* is an angiosperm dioecious plant, which is used for cash income, as a bittering agent for the preparation of local alcoholic beverage and medicinal value. *Rhamnus prinoide* is a dense shrub or sometimes appears as small tree. It is spineless and evergreen. It is one of the two representative species with *Rhamnus staddo* of the family *Rhamnacaea* that are only found in Africa (Phillips, 1994).

The closely related species *R.staddo* A. Rich. (Vernacular name, tsedo) occurs only in the wild at the edges of Montana forest in wooded and scrub land in many parts of Ethiopia. *R.prinoides* is cultivated on a large scale as a field crop. It is also grown in home gardens (Fullas, 2003).

2.3.2. Malt (Bikil)

Bikil is prepared from malted cereals which may be wheat 0at or barley. The cereal is steeped in water for about two days. The second day the water is drained off and the cereal is left packed in a container for one day to allow germination.

The germinated grain is transferred in to a wide tray to allow the germinated grain to lock with each other. Then grain is smoked, sun dried and grounded to coarse powder (Nigussie, 2008).

Germination of cereal grains is initiated by water and its successful completion is signaled by the emergence of the developing rokots and shoots.

Following the uptake of water, hormonal signals, probably released from the embryo are believed to result in the synthesis of hydrolytic and other enzymes by the aleurone cells and scutellum (Eglin and Oloyede, 2006).

Malt is germinated cereal grain that has been dried in a process known as malting; the grain is made to germinate by soaking in water and is then halted from germinating further by drying with hot air. Malted cereal grains have combination of enzymes, α-amylase and β-amylase, dextrinase, protease, phosphatase, peptidase and others enzymes commonly called in combination malt diastase. Malt diastase is derived from scutellum, aleuron layers and the starchy endosperm of germinated seed to hydrolyze starch molecules into simple sugar to nourish the growing seedling (Egwim and Oloyede, 2006).

During germination and early seedling development, hydrolytic enzymes; α-amylase, β-amylase limit dextrinase and α-glucosidase enzymes convert insoluble starch granules of the endosperm into glucose (Charalampopoulos *et al.*, 2003). Alpha amylase hydrolyzes alpha linkages in the two types of starch forms, αamylose and amylopectin, to yield a mixture of glucose and free maltose.

Alpha amylose consists of long chains in which all the glucose units are bound in α-(1-4) linkages and the amylopectin is highly branched with back bone linkage of glycosidic α-(1-4) and the branch points are α-(1-6) glycosidic linkage.

2.3.3. Honey

Honey is a natural product, mainly composed of a complex mixture of carbohydrates and other minor substances, such as organic acids, amino acids, protein's, minerals, vitamins, and lipids (Finola *et al*., 2007). Most honey contains high sugar content and low water content which is extremely unsuitable for microorganisms.

Honey has been highly appreciated as an alimentary product and has been largely used, since ancient times, either preventing or curing infirmity and illness or in cosmetic manufacturing.Honey is a sugary substance obtained from the nectar of the flowers or from the secretions which come from or lie on the living parts of the plant and which honey bees' crop, transform and combine with their own specific substances and store in the honeycomb of the beehive(Saklani and Kumar 2021).

Honey is a product extremely rich in sugars of which glucose and fructose are outstanding. It also possesses vitamins, mineral salts and enzymes.

Honey, however and despite having a high tenor in sugars and consequently a reduced water activity, an acid pH, and bactericidal substances (H_2O_2 and inhabits), it frequently suffers fermentations driven by yeasts, which make this product inappropriate for consumption. Fermentation is an irreversible phenomenon that can run in honey mainly during storage, causing significant economic losses. In such a case, honey presents a characteristic odor, increasing acid flavor and gas bubbles (Carvalho *et al.*, 2005). The microbes of concern in honey are primarily yeasts and spore-forming bacteria. Standard industry practices control yeast growth. Bacterial spores, particularly those in the *Bacillus genus*, are regularlyfound in honey(Bevilacqua, Corbo *et al*. 2024).

2.4. Yeasts

Yeasts are eukaryotic micro-organisms classified in the kingdom Fungi, with about 1,500 species currently described (Kurtzman and Fell, 2006). Yeasts do not form an exact taxonomic or phylogenetic grouping. At present it is estimated that only 1% of all yeast species have been described (Kurtzman and Piškur, 2006). The term "yeast" is often taken as a synonym for *S. cerevisiae* (Kurtzman, 1994). But the phylogenetic diversity of yeasts is shown by their placement in both divisions Ascomycota and *Basidiomycota.*

Yeast is an important industrial microorganism and has important applications in the fields of food, feed, medicine and environmental management (huang *et al*, 2020).

Yeasts are predominant microorganisms in most of the traditional fermented foods and beverages such as burukutu, seera, chhang, fufu, tape, laochao, etc. (Bhalla, 2017). The traditional fermented foods and beverages in Africa have social, religious cultural and nutritional importance. yeast utilization as sources of food ingredients and additives, such as flavor’s, color’s, antioxidants and vitamins could allow in malnutrition fighting in developing countries (Hull *et al.*, 2014; Bhalla, 2017).

The budding yeasts ("true yeasts") are classified in the *order Saccharomycetales*. Yeasts dominate fungal diversity in the oceans (Bass, *et al.*, 2007). Most reproduce asexually by budding, although a few do so by binary fission.

Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudo hyphae, or false hyphae as seen in most molds (Kurtzman and Fell, 2005).

Yeast size can vary greatly depending on the species, typically measuring 3-4 µm in diameter, although some yeast can reach over 40 µm (Walker, *et al.*, 2002).

The yeast species *Saccharomyces cerevisiae* has been used in baking and fermenting alcoholic beverages for thousands of years. It is also extremely important as a model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms. Researchers have used it to gather information about the biology of the eukaryotic cell and ultimately human biology (Ostergaard, *et al*., 2000). Other species of yeast, such as *Candida albicans*, are opportunistic pathogens and can cause infections in humans. Yeasts have recently been used to generate electricity in microbial fuel cells, and produce ethanol for the biofuel industry.

Yeasts convert sugars (through a process known as fermentation) into alcohol and carbon dioxide. This trait is what endears yeasts to winemakers, brew 18 masters and bread bakers. In the making of wine and beer, the yeasts' manufacture of alcohol is desired and necessary for the final product; and carbon dioxide is what makes beer and champagne effervescent. The art of bread making needs the carbon dioxide produced by yeast in order for certain dough's to rise(Ali, Shehzad *et al*. 2012).

2.4.1. Yeast as food ingredients

Yeast is one of the first domesticated microorganisms used in fermented foods production, foods and food ingredients in human alimentation in Africa (Mogmenga *et al.*, 2017). The role of yeasts in foods and beverages production is known for a long time.

Several types of yeasts including *Saccharomyces cerevisiae, Candida tropicalis, Candida utilis, Rhodotorula glutinis, Sporobolomyces patagonicus, Sporobolomyces roseus, Rhodotorula mucilaginosa, Yarrowia lipolytica, Kluyveromyces lactis, Geotrichum candidum* are used for production of values added products (Ouedraogo *et al*., 2017).

Application of yeast starters has become a standard practice in the industrial fermentation for food and beverages and other products such as organic acids, amino acids, vitamins, lipids, carotenoid, glycerol (Türker, 2014). Most of those compounds produced by yeasts are used in human foods and food ingredients. Several studies carried out showed that those value – added products are produced by yeasts using raw materials of plant as well as animal origin. Indeed, mangoes, molasses, potatoes, sorghum, wheat, rice, paper industrial wastes were used for value – added products production (Somda *et al*., 2017).

Saccharomyces cerevisiae is the most frequently involved yeast species in the production of yeast foods and food ingredients (Bhalla, 2017). Using yeasts for value added products production is related to the fact that yeasts are able to ferment a broad range of carbon substrate and generate secondary metabolites which can be used in several fields such as food, cosmetic, and pharmaceutical industries.

2.4.2 Yeasts as food

Yeasts are dominant microorganisms in most of the traditional fermented foods and beverages such as burukutu, Merissa, bhatooru, seera, chhang, fufu, tape, ogi, puto, dosa, idli, papdam, kecap, laochao, warri, etc (Bhalla, 2017). In these traditional foods, yeast either alone or in association with bacteria and mould has substantial influence on taste, aroma, texture and nutritional value of the fermented products. Yeasts have been cultivated also as rich sources of protein, minerals, vitamins (particularly B vitamins), and other nutrients for humans and animals (Ouedraogo *et al*.,2017).

Several yeast species have been used for biomass production, including *Candida utilis,* other *non-methylotrophic Candida spp., Saccharomycopsis fibuligera, Kluyveromyces spp., and Saccharomyces cerevisiae* (Ouedraogo *et al*., 2017). Methylotrophic, ethanol-utilizing, and fat and hydrocarbon-utilizing yeasts including species of *Candida, Ogataea, Pichia,* and *Trichosporon* have also been used for biomass production (Türker, 2014). Thus, yeasts play very important role in improving the nutrition and socio-cultural life of people living in rural and tribal areas of various countries across the world.

2.4.3. Nutritional quality of yeasts

Yeasts are a great nutritional value and can contain more than 60% of proteins and 15% of lipids (Ouedraogo *et al*., 2017). Obtained 54.8% of protein with *Candida utilis* in single cell Protein (SCP) production using tubercles wastes as a substrate. The high rate of protein in yeasts show that yeasts can be used like food complement to increase nutritional value of certain food par example cereal food. Amino acids analysis from yeasts SCP shows that yeasts contain high viral fields such as food, cosmetic and pharmaceutical industries (Türker 2014).

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted at Kobo and Robit town. Kobo town is located 570km away from Addis Abeba in the northern part of Ethiopia and the town belongs to longitude of 12°09′N and 39°38′E latitudewith an elevation of 1468 meters above sea level and Robit town was found in 12°0'47.32"N,39°37'42.91"E. A total of 301,102 total population were counted in this district, amog this151,541 wre male and 149,561 were females found in both kobo and robit town. The majority of the inhabitants practiced Ethiopian Orthodox Christianity, with 82.88% reporting that as their religion, while 16.5% of the population said they were Muslim.The majority of the population practiced Ethiopian Orthodox Christianity with 83.2% reported to profess this belief, while 16.72% of the population said they were Muslim (CSA,2022).

3.2. Study Design and Period

A cross-sectional study design was implemented to investigate the dominant yeast species present in traditionally prepared Tella and Tej, two traditional Ethiopian alcoholic beverages. The study was conducted over a period spanning from February 2023 to March 2024.

3.3. Source of Population

The population for this study consisted of vendors who sell Tella and Tej in the Raya Kobo district. A purposive random sampling technique was employed to ensure a representative selection of participants. All available vendors of Tella and Tej were included in the study, allowing for a comprehensive assessment of the yeast species associated with these traditional beverages. This approach facilitated the collection of diverse samples reflective of the local fermentation practices.

3.4. Sample Size

Samples of Tella and Tej were collected from Kobo and Robit towns within the Raya Kobo district, as outlined in the study. A purposive sampling technique was employed, wherein all available vending houses selling these traditional beverages were selected for the study, ensuring a representative sample of the local production (Cochran, 1977). A total of 30 samples were collected, with 15 samples each for Tella and Tej from both Robit and Kobo. Each sample consisted of 250 ml, collected in sterile screw-capped bottles to prevent contamination and maintain sample integrity (APHA, 2017).

Following collection, the samples were immediately stored in a refrigerator at 4°C to preserve their quality and prevent microbial growth until further processing could be conducted (FDA, 2020).

3.5. Sample Collection

A purposive random sampling technique was employed to collect samples from vending houses (Cochran,1977). Accordingly, 250 milliliters of samples were aseptically obtained from the storage containers of thirty households of Tella and Tej vendors. The samples were placed into sterilized bottles and labeled appropriately according to their sampling site and code (APHA, 2017). The collected samples were immediately placed in an ice box and transported to the Woldia University laboratory within three hours for analysis. Prior to collection, each sample was agitated to achieve a homogeneous mixture, ensuring consistency in the analysis (FDA, 2020).

3.6. Data Collection Techniques

A structured questionnaire was utilized to gather the necessary information from respondents. The questionnaire was designed to include both close-ended and open-ended questions, covering key aspects such as knowledge about the beverages, production practices, handling methods, and other relevant factors. Additionally, an observation checklist was incorporated to record observable practices and conditions related to beverage handling and preparation. To ensure the reliability and validity of the data, all completed questionnaires were thoroughly checked for accuracy and completeness (Fowler, 2014).

To facilitate better understanding and participation among the study participants, the questionnaire and observation checklist were initially prepared in English and subsequently translated into Amharic, the local language of the study area (WHO, 2007). This approach was adopted to minimize language barriers and ensure that the respondents could provide accurate and meaningful responses.

The translation process was carefully conducted to maintain the integrity and consistency of the questions, and the translated tools were pretested to confirm their clarity and appropriateness for the target population (Brislin, 1970).

3.7. Isolation of Yeast from Tella and Tej

Yeast isolation from Tella and Tej was conducted using standardized microbiological procedures. Fresh samples of Tella and Tej were collected in sterile containers and shaken thoroughly to ensure homogeneity. Serial dilutions were prepared by transferring 1 mL of each sample into 9 mL of sterile peptone water (0.1%), with dilutions repeated up to 10^{-6} (APHA, 2017). From each dilution, 0.1 mL was plated onto Yeast Peptone Dextrose (YPD) agar and Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol to inhibit bacterial growth. The samples were spread evenly using a sterile spreader and incubated at 25-30°C for 24-48 hours. Yeast colonies, identified by their smooth, shiny, creamy, or white appearance, were streaked onto fresh media for purification and incubated under the same conditions (Kurtzman et al., 2011). Yeast isolates from Tej and Tella were designated with the letters WUJY (Woldia University Tej Yeast) and WUTY (Woldia University Tella Yeast), respectively, followed by consecutive Roman numerals.

Purified yeast cultures were preserved by transferring to agar slants for short-term storage at 4°C or in 20-30% glycerol at -80°C for long-term storage. Morphological confirmation was performed microscopically using Gram staining and lactophenol cotton blue, while biochemical tests were conducted for species identification (Kurtzman et al., 2011).

3.8. Biochemical Characterization of the Yeast

The biochemical characterization of yeast isolates from Tella and Tej was conducted using a series of standardized tests to evaluate their metabolic and physiological properties. These tests were performed to identify and differentiate yeast species based on their ability to utilize specific substrates and tolerate varying environmental conditions (Kurtzman et al., 2011).

3.8.1. Citrate test

The citrate utilization test was carried out to determine the ability of the yeast isolates to use citrate as a sole carbon source. The isolated colonies from both Tella and Tej were inoculated onto Simmons' citrate agar, which was prepared by dissolving the medium in 1 L of distilled water.

The inoculated plates were incubated at 37°C for 48 hours. A change in the color of the medium from green to blue indicated a positive result, confirming the ability of the isolates to metabolize citrate (MacFaddin, 2000).

3.8.2. Urease test

The urease test was performed to assess the ability of the yeast isolates to hydrolyze urea into ammonia and carbon dioxide. The isolates were inoculated onto urea agar medium, prepared by dissolving 20.30 g/L of urea in distilled water. The plates were incubated at 37°C for 48 hours. A positive result, indicated by a color change to pink or red, demonstrated the presence of urease activity (Barnett et al., 2000).

3.8.3. Fermentation of carbohydrates

The carbohydrate fermentation test was conducted to evaluate the ability of the yeast isolates to ferment different sugars as carbon and energy sources. The isolates were inoculated into fermentation broth containing various sugars, such as glucose, sucrose, lactose, and maltose. The tubes were incubated at 48°C, and the production of gas or acid was observed to determine the fermentation capabilities of the isolates (Harrigan & McCance, 1976).

3.8.4. Hydrogen sulphide test

The hydrogen sulphide (H_2S) production test was performed to determine the ability of the yeast isolates to reduce sulfur compounds to H_2S. The isolates were inoculated onto media containing sulfur compounds, such as peptone iron agar or triple sugar iron agar. The plates were incubated at 37°C for 48 hours. The formation of a black precipitate indicated a positive result, confirming the reduction of sulfur to H_2S (MacFaddin, 2000).

3.8.5. Ethanol tolerance test

The ethanol tolerance test was conducted to assess the ability of the yeast isolates to grow in the presence of varying ethanol concentrations. The isolates were inoculated into 10 mL of liquid Yeast Extract Peptone Dextrose (YEPD) broth containing ethanol at concentrations of 5% and 10%. The inoculated broths were incubated at 30°C for 48 hours, and growth was observed to determine the ethanol tolerance levels of the isolates (Kurtzman et al., 2011).

3.9. Physico-Chemical characteristics of Yeast Isilates

The pH, conductivity, and total dissolved solids (TDS) of the Tella and Tej samples were measured using a digital pH, conductivity, and TDS meter (model: Adwa AD8000 pH/Conductivity/TDS). Prior to the measurements, the meter was calibrated with a standard solution to ensure accuracy.

The electrode of the meter was dipped into the samples, and the readings for pH, conductivity, and TDS were recorded. This method ensured that the physico-chemical properties of the samples were accurately determined, as supported by standard protocols for such analyses (APHA, 2017).

3.10. Heavy Metal Tolerance Assay of Yeast Isolated Tella and Tej

The resistance of yeast isolates to heavy metals (lead, cadmium, and copper) was assessed using growth inhibition studies on YEPD agar plates, following established methods (Kavamura & Esposito, 2010; Gavrilescu, 2022). YEPD agar, suitable for diverse yeast species was supplemented with lead nitrate ($Pb(NO_3)_2$), cadmium chloride ($CdCl_2$), and copper sulfate ($CuSO_4$) at concentrations of 50, 100, and 200 mg/L, consistent with prior studies. Fresh yeast cultures were inoculated onto the plates using sterile techniques to ensure uniform growth and minimize contamination (Barnett et al., 2000).

Plates were incubated at 30°C for 72 hours, optimal for yeast growth and inhibition zones indicating metal-induced growth suppression were measured post-incubation using a ruler or caliper (Gadd, 1993). Isolates with no or small inhibition zones were classified as resistant, while those with larger zones were deemed sensitive, reflecting their tolerance to heavy metal stress.

3.11. Intricsic Antibiotic Resistance Test of the Yeast Isolates

The intrinsic antibiotic resistance of yeast isolates was evaluated using the disk diffusion method (CLSI, 2020). YEPD agar was prepared as the growth medium, and fresh yeast cultures were adjusted to 0.5 McFarland units to ensure consistent inoculum density (Odds, 1988). Antibiotic disks containing the following concentrations were used: fluconazole (25 µg), amphotericin B (10 µg), ketoconazole (15 µg), nystatin (100 µg), and chloramphenicol (30 µg). The disks were placed on the inoculated plates and incubated at 30°C for 24–48 hours (Pfaller et al., 2007). After incubation, inhibition zones were measured, and the yeast isolates were classified as resistant (-), intermediate (++), or susceptible (+++) based on the zone size.

Isolates with no or small inhibition zones were classified as resistant (-), those with moderate inhibition zones as intermediate (++), and those with large inhibition zones as susceptible (+++).

3.12. Data Analysis

After the data had been collected, it was cleaned manually to ensure accuracy and consistency. Both qualitative and quantitative data were analyzed statistically.

The qualitative data obtained from the hygiene practices questionnaire were processed, and the frequency and percentage of responses were calculated. These results were then partitioned according to the locations from which the data were collected. For the quantitative data, bacterial counts were analyzed and presented as the mean ± standard deviation (SD) of log CFU/mL (colony-forming units). This statistical approach was used to compare differences among the mean contamination levels of yeast species isolates. The analysis provided a comprehensive understanding of the data, ensuring that variations and trends were accurately identified and interpreted.

4. RESULTS AND DISCUSSION

4.1. Socio Demographic Characteristics

The socio-demographic characteristics of Tella and Tej vendors were analyzed using data presented in Table 1, focusing on gender, age, educational status, marital status, and religion. The sociodemographic characteristics of the respondents were analyzed in detail. All 30 participants (100%) were found to be female, with no male respondents recorded. Regarding age distribution, the largest proportion of respondents (16 individuals, 64%) were above 44 years old. A total of 10 respondents (33.7%) were between the ages of 31 and 43, while the remaining 4 participants (13.3%) were within the 18–30 age group.

Educational status was also assessed. Among the respondents, 6 individuals (20%) were identified as illiterate. A total of 10 participants (33.3%) had attained basic education, while 8 respondents (26.7%) had completed elementary education. Secondary education was reported among 3 individuals (10%), and an equal proportion of 3 respondents (10%) held a diploma. The marital status analysis showed that the majority of respondents, 17 individuals (56.7%), were widowed. Additionally, 7 respondents (23.3%) were unmarried, while 6 participants (20%) were married.Regarding religious affiliation, all 30 respondents (100%) identified as Orthodox Christian, with no participants belonging to other religious groups.

TABLE 1.SOCIODEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

	Characteristics	Frequency	Percent
Gender	Male	-	-
	Female	30	100
Age level	18-30	4	13.3
	31-43	10	33.7
	Above 44	16	64
Educational status	Illiterate	6	20
	Basic education	10	33.3
	Elementary education	8	26.7
	Secondary education	3	10
	Diploma	3	10
Marital status	Married	6	20
	Un married	7	23.3
	Widowed	17	56.7
Religion	Muslim	-	-
	Orthodox	30	100

4.1. 2. Knowledge of vendors on tej and tella preparation

The knowledge of vendors regarding the preparation of Tej and Tella was analyzed based on data presented in Table 2, focusing on key aspects of their understanding and practices in beverage preparation. The knowledge of respondents regarding the preparation of Tella and Tej was analyzed. All 30 participants (100%) demonstrated familiarity with the preparation methods of these traditional beverages.

The majority of respondents (28 individuals, 93.3%) reported that Tella and Tej were prepared at home, while only 2 participants (6.7%) stated that these beverages were not prepared in their households.

Hygienic practices related to fermentation were also evaluated. All respondents 30(100%) confirmed that traditional fermenters cleaned their containers using Grawa and Weira. Additionally, all participants (100%) reported that fermenters ensured the safety of the bermel barrel during the fermentation process.

The socio-economic significance of Tella fermentation was universally acknowledged by all respondents (100%). It was highlighted that Tella serves multiple purposes, including being a family drink, a beverage consumed during holidays and social gatherings, a key element in wedding ceremonies, and a source of income for many households.The duration of Tella fermentation process varied among respondents. A total of 6 participants (20%) reported that Tella was ready for consumption in 6 days, while the majority, 21 respondents (70%), indicated that the process took 7 days. Only 3 individuals (10%) stated that the fermentation process lasted for 8 days.

Table 2.Knowledge of Respondents on Tella and Tej prepation

ITEMS		*YES*		*NO*	
		Frequency (N)	Percent (%)	Frequency (N)	Percent (%)
Does the vender know about preparation of tej and Tella?		30	100 %	0	0
Is the traditional beverages prepared in their home?		28	93.3%	2	6.7
Does the traditional fermenter wash their container, by Grawa andWeira?		30	100 %	0	0
Does the fermenter keep the safety of bermelBarrel?		30	100 %	0	0
What is socio	family drinking for	30	100%	0	0
economic value of	holidays for social	30	100%	0	0
Tella fermentation?	works for weeding ceremonies	30	100%	0	0
	for source of income	30	100%	0	0
Duration of Tella	6 days	6	20%		80
process from preparation	7 days	21	70%		20
to consumption?	8 days	3	10%		90

4. 1. 3. Physico-chemical parameters of tella and tej samples

The physico-chemical characteristics of the *Tella* and *Tej* samples were analyzed based on the data presented in Table 3. The physico-chemical properties of *Tella* and *Tej* samples were analyzed based on pH, total dissolved solids (TDS), electrical conductivity (EC), and temperature. The results revealed significant differences between the two traditional Ethiopian beverages, particularly in acidity and dissolved solid concentrations.The pH values of *Tella* isolates ranged from 4.49 to 4.94, indicating a moderately acidic environment.

The highest pH value (4.94) was recorded in isolates WUTY3, WUTY4, WUTY11, and WUTY13, while the lowest pH (4.49) was observed in isolates WUTY1, WUTY5, WUTY8, and WUTY12.

In contrast, the *Tej* isolates exhibited lower pH values, ranging from 3.63 to 3.94. The most acidic samples (pH 3.63) were WUJY9, WUJY10, and WUJY14, whereas WUJY1, WUJY2, and WUJY11 showed the highest pH (3.94). These results indicated that *Tej* was more acidic than *Tella*, suggesting a more advanced fermentation process or the influence of additional fermentation agents such as *gesho* (*Rhamnus prinoides*), which may enhance acidity. The lower pH in *Tej* was expected to contribute to its microbial stability by inhibiting the growth of certain spoilage organisms.The total dissolved solids (TDS) values of *Tella* samples ranged between 1256 and 1405 µS, while the corresponding electrical conductivity (EC) values varied from 1250 to 1400 µS/cm. The highest TDS concentration (1405 µS) was observed in WUTY1, WUTY5, WUTY8, and WUTY12, while the lowest TDS (1256 µS) was recorded in WUTY2, WUTY6, WUTY10, and WUTY15. Similarly, *Tej* samples exhibited lower TDS values, ranging from 1060 to 1210 µS, with corresponding EC values between 1045 and 1142 µS/cm. The lowest TDS (1060 µS) was recorded in WUJY9, WUJY10, and WUJY14, whereas the highest (1210 µS) was found in WUJY10 and WUJY7. The lower TDS and EC values in *Tej* suggested that it contained fewer dissolved solids compared to *Tella*.

This difference could be attributed to the ingredients used in the fermentation process, where *Tella* is typically prepared from grains such as barley, maize, or sorghum, whereas *Tej* is based on honey fermentation. The higher TDS in *Tella* indicated a greater concentration of dissolved organic and inorganic compounds, which might contribute to its richer taste and denser composition.

The temperature of the samples was recorded to assess their storage and fermentation conditions.

Tella samples showed temperature values ranging from 22.7°C to 23.7°C, while *Tej* samples exhibited a similar range between 22.9°C and 23.5°C. The highest temperature for *Tella* (23.7°C) was recorded in isolates WUTY3 and WUTY4, whereas the lowest (22.7°C) was observed in WUTY15 and WUTY10. Similarly, for *Tej*, the maximum temperature (23.5°C) was noted in WUJY10 and WUJY7, while the lowest (22.9°C) was recorded in WUJY5, WUJY8, and WUJY13.

The narrow temperature range between the two beverages suggested that they were analyzed under relatively similar conditions, minimizing external temperature influences on fermentation and microbial activity.

The analysis of *Tella* and *Tej* samples demonstrated clear differences in their physico-chemical properties. *Tej* was found to be more acidic than *Tella*, which could enhance its preservation properties and impact its sensory characteristics. Additionally, *Tella* contained a higher concentration of dissolved solids, as reflected in its higher TDS and EC values. These findings indicated that *Tella* was likely to have a denser composition, while *Tej* exhibited a lighter consistency with greater acidity.

The temperature variations were minimal, ensuring reliable comparisons between the two beverages. These results provided valuable insights into the fermentation characteristics and quality attributes of *Tella* and *Tej*, which are essential for optimizing their production and preservation.

Table3. Physico-chemical Characteristics of Tella and Tej

Isolates from Tella	pH	Isolates from Tej	pH	TDS (µS) Tella	EC (µS/cm) Tella	Temp (°C) Tella	TDS (µS) Tej	EC (µS/cm) Tej	Temp (°C) Tej
WUTY3, WUTY4, WUTY11, WUT13	4.94	WUJY3, WUJY4, WUJY6, WUJY15	3.89	1349	1375	23.7	1136	1100	23.3
WUT2, WUTY6, WUTY10, WUTY15	4.56	WUJY9, WUJY10 , WUJY14	3.63	1256	1250	23.1	1060	1050	23.7
WUTY1, WUTY5, WUTY8, WUTY12	4.49	WUJY5, WUJY8, WUJY13	3.82	1405	1400	23.3	1062	1049	22.9
WUTY7, WUTY14, WUTY9	4.63	WUJY1, WUJY2, WUJY11	3.94	1279	1275	22.9	1070	1055	22.9
WUTY11, WUTY13	-	WUJY15 , WUJY5	-	1340	1335	22.8	1078	1045	23.3
WUTY15, WUTY10	-	WUJY12 , WUJY8	-	1278	1275	22.7	1190	1075	23.1
WUTY12	-	WUJY10 , WUJY7	-	1260	1250	23.4	1210	1142	23.5

TDS = total disolved substance, µS = microsiemens, PH=,

TEMP=Temprature,EC=Electrical conductivity

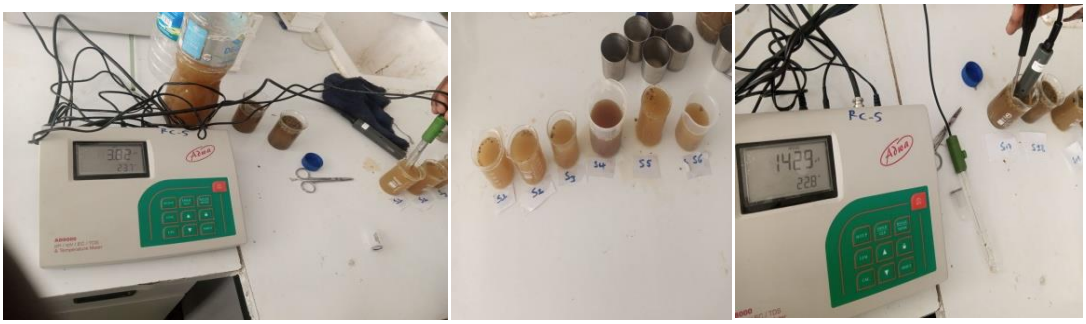

Figure1.Sample with TDS, PH and Temprature

4. 1. 4. Identification of yeasts based on their morphological characteristics

The yeast isolates obtained from *Tella* and *Tej* samples were analyzed based on colony morphology, including colony form, color, elevation, surface texture, margin characteristics, and budding patterns based on table 5. These characteristics provided insights into the dominant yeast species present in each beverage. Concerning Yeast Isolates fromTella**,** The isolates from *Tella* exhibited circular colony forms with smooth surfaces and entire margins, indicating uniform growth. The majority of the isolates (WUTY1, WUTY2, WUTY4, WUTY6, WUTY7, WUTY8, WUTY10, WUTY12, WUTY13, WUTY14, and WUTY15) were observed to have cream-colored colonies with a convex elevation. These isolates were identified as *Saccharomyces cerevisiae*, a yeast species commonly associated with alcoholic fermentation.

The budding pattern was found to be multilateral, with cells appearing round to oval. This characteristic suggested that *Saccharomyces cerevisiae* played a major role in the fermentation of *Tella*, contributing to ethanol production and flavor development.Another group of *Tella* isolates (WUTY3, WUTY5, WUTY9, and WUTY11) exhibited white to cream-colored colonies, maintaining the circular form, smooth surface, and entire margin. These isolates were identified as *Zygosaccharomyces bailii*, which was observed to bud in a multilateral fashion, with cells appearing ellipsoid, either singly or in pairs. This yeast species is known for its high resistance to acidic environments and preservatives, suggesting its potential role in enhancing the stability of *Tella*.Regarding Yeast Isolates from *Tej*,The isolates from *Tej* also displayed circular colony forms with smooth surfaces and entire margins, similar to those observed in *Tella*.

The majority of the isolates (WUJY1, WUJY2, WUJY3, WUJY7, WUJY8, WUJY10, WUJY11, WUJY12, WUJY13, and WUJY14) were identified as *Saccharomyces cerevisiae*. These colonies were cream-colored with convex elevations, and the yeast cells exhibited multilateral budding, appearing generally round to oval. This finding suggested that *Saccharomyces cerevisiae* was also the dominant fermenting yeast in *Tej*, contributing to alcohol production and flavor enhancement.

A distinct group of *Tej* isolates (WUJY5 and WUJY6) exhibited a unique budding pattern, characterized by bipolar budding with apiculate (lemon-shaped) cells. These isolates were identified as *Hanseniaspora* species. The presence of *Hanseniaspora* species suggested an important role in the early stages of *Tej* fermentation, as these yeasts are known to produce fruity aroma compounds and contribute to flavor complexity.

Additionally, isolates WUJY4, WUJY9, and WUJY15 were found to belong to *Pichia pastoris*. These colonies were cream-colored with a convex elevation and displayed multilateral budding. The yeast cells appeared oval to cylindrical, indicating their morphological distinction from other isolates. *Pichia pastoris* is often associated with fermentation and can influence the sensory characteristics of alcoholic beverages through enzyme production and secondary metabolite formation.

The analysis of yeast isolates from *Tella* and *Tej* revealed that *Saccharomyces cerevisiae* was the dominant species in both beverages, playing a central role in fermentation. However, variations in yeast species were observed, with *Zygosaccharomyces bailii* present in *Tella* and *Hanseniaspora* and *Pichia pastoris* detected in *Tej*. The differences in yeast composition suggested that *Tej* underwent a more complex fermentation process, influenced by multiple yeast species contributing to its unique flavor and characteristics. These findings highlighted the microbial diversity in traditional Ethiopian fermented beverages and their potential impact on product quality.

Table 4. Morphological characteristics of yeast cell colonies isolated from tella and tej

Sample type	Isolates	Colony Form	Colony Color	Colony Elevation	Colony Surface	Colony Margin	Budding	**Yeast Species**
Tella	WUTY1,2,4,6,7,8,10,12,13,14,15	Circular	Cream	Convex	Smooth	Entire	Multilateral round to val.	*Saccharomyces cerevisiae*
	WUTY3,5,9,11	Circular	White to cream	Convex	Smooth	Entire	Multilateral ellipsoid, singly or in pairs. O	*Zygosaccharomyces bailii*
	WUJY5,6	Circular	Cream	Convex	Smooth	Entire	Bipolar budding; cells are apiculate (lemon-shaped).	*Hanseniaspora species*
Tej	WUJY4,9,15	Circular	Cream	Convex	Smooth	Entire	Multilateral budding; cells are oval to cylindrical.	*Pichia pastoris*
	WUJY1,2,3,7,8,10,11,12,13,14	Circular	Cream	Convex	Smooth	Entire	Multilateral budding; cells are generally round to oval.	*Saccharomyces cerevisiae*

Figure 2.Cultured yeast species

4.1. 5. Biochemical characteristics of the isolated yeast species

The biochemical characteristics of the isolated yeast species were examined through various carbohydrate fermentation tests and enzymatic activities according to table 6. The results provided valuable insights into the metabolic capabilities and potential roles of these yeast species in the fermentation process.

The ability to ferment different carbohydrates was assessed for *Saccharomyces cerevisiae, Zygosaccharomyces bailii, Hanseniaspora* species, and *Pichia pastoris*. Glucose was positively fermented by all yeast species, indicating its universal utilization as a primary energy source. Similarly, sucrose and maltose were metabolized by *Saccharomyces cerevisiae, Zygosaccharomyces bailii,* and *Pichia pastoris*. However, the fermentation of sucrose and maltose by *Hanseniaspora* species was found to be variable, suggesting that different strains may exhibit differing capabilities in utilizing these sugars.

Lactose was not fermented by any of the yeast species, indicating the absence of lactase activity. Raffinose fermentation was observed to be species-dependent, with *Zygosaccharomyces bailii* showing positive results, *Saccharomyces cerevisiae* displaying variable fermentation, and *Hanseniaspora* species and *Pichia pastoris* exhibiting negative results. The ability to ferment trehalose was found to be consistent in *Saccharomyces cerevisiae, Zygosaccharomyces bailii,* and *Pichia pastoris*, whereas *Hanseniaspora* species exhibited variable results.

Table 6. Biochemical characteristics of Isolated Yeast Species from tej and tella from raya kobo in 2025

Test / Carbohydrate	*Saccharomyces cerevisiae*	*Zygosaccharomyces bailii*	*Hanseniaspora species*	*Pichia pastoris*
Glucose	Positive	Positive	Positive	Positive
Sucrose	Positive	Positive	Variable	Positive
Maltose	Positive	Positive	Variable	Positive
Lactose	Negative	Negative	Negative	Negative
Raffinose	Variable	Positive	Negative	Negative
Trehalose	Positive	Positive	Variable	Positive
Starch	Negative	Negative	Negative	Negative
Dextrin	Negative	Negative	Negative	Negative
Xylose	Negative	Negative	Variable	Negative
H_2S Production	Negative	Negative	Variable	Negative
Citrate Utilization	Negative	Negative	Negative	Negative
Urease Activity	Negative	Negative	Negative	Negative
Ethanol Tolerance	High	High	Low	Moderate

The utilization of complex carbohydrates such as starch and dextrin was not observed in any of the isolated yeast species, as negative results were recorded across all tested strains. This indicated that none of these yeasts possessed the necessary enzymatic pathways to hydrolyze and metabolize these polysaccharides. Similarly, xylose fermentation was found to be negative in *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Pichia pastoris*, while *Hanseniaspora* species exhibited variable results, suggesting partial xylose metabolism in some strains.

Hydrogen sulfide (H_2S) production was found to be negative for *Saccharomyces cerevisiae, Zygosaccharomyces bailii,* and *Pichia pastoris*, while *Hanseniaspora* species exhibited variable results, indicating that some strains had the potential to produce H_2S. Citrate utilization and urease activity were observed to be negative in all yeast species, suggesting that these yeasts did not metabolize citrate as a sole carbon source and lacked urease enzyme activity.

Variations in ethanol tolerance among the yeast species were observed. *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* demonstrated high ethanol tolerance, supporting their roles as dominant fermentative yeasts in alcoholic beverage production. In contrast, *Hanseniaspora* species exhibited low ethanol tolerance, suggesting their primary involvement in the early stages of fermentation. *Pichia pastoris* displayed moderate ethanol tolerance, indicating its ability to survive under fermentative conditions but not as efficiently as *Saccharomyces cerevisiae* or *Zygosaccharomyces bailii*.

The biochemical characterization of the isolated yeast species confirmed their distinct metabolic properties and potential contributions to fermentation. *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* were identified as strong fermenters with high ethanol tolerance, making them essential for alcohol production. *Hanseniaspora* species, despite their limited ethanol tolerance, were found to exhibit unique metabolic characteristics that may contribute to flavor development. *Pichia pastoris* was identified as a moderate fermenter with distinct carbohydrate utilization patterns. These findings provided essential insights into the microbial composition of traditional Ethiopian fermented beverages and their potential impact on product quality and fermentation efficiency.

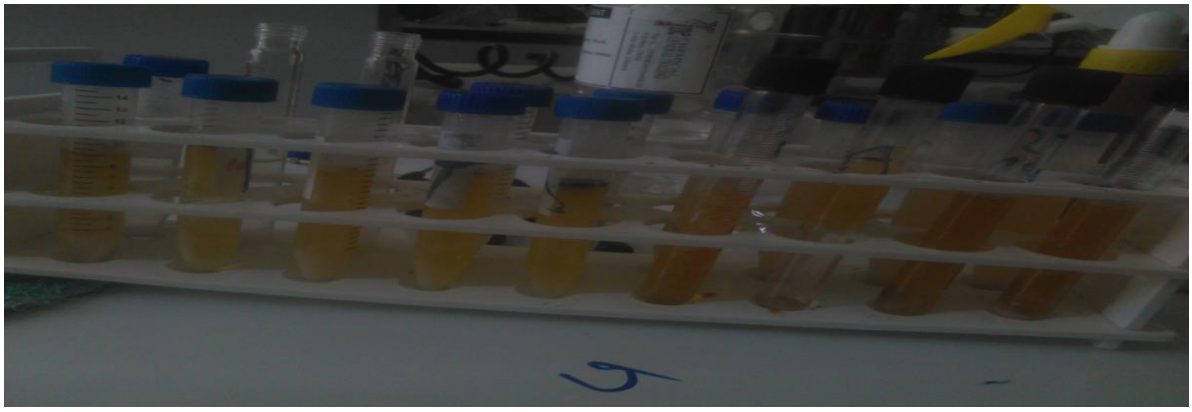

Figure 3. Biochemical Test

4.1. 6. Mean CFU/ml of the isolated yeast species

The mean maximum and minimum counts of the isolated yeast species in *Tella* and *Tej* were analyzed, along with their respective log10(Li, Wang et al.)10 values, to determine the prevalence and distribution of each species. The results provided insights into the microbial composition of these traditionally fermented beverages.Among the identified yeast species, *Saccharomyces cerevisiae* was observed to have the highest mean counts in both *Tella* and *Tej*. The maximum recorded mean count in *Tella* was 5.2×10^6 cfu, while the minimum was 5×10^5 cfu. In *Tej*, the maximum count reached 4×10^6 cfu, whereas the minimum was found to be 4×10^4 cfu. These high counts suggested that *Saccharomyces cerevisiae* was the dominant yeast species, playing a crucial role in the fermentation process. The mean log10(Li, Wang et al.)10 values were recorded as 6.72 for *Tella* and 6.60 for *Tej*, further confirming the significant presence of this species in both beverages.

The population of *Zygosaccharomyces bailii* was recorded at lower levels than *Saccharomyces cerevisiae*. In *Tella*, the maximum mean count was observed at 4×10^4 cfu, while the minimum was 3.2×10^3 cfu. Similarly, in *Tej*, the maximum and minimum mean counts were 3×10^4 cfu and 2×10^3 cfu, respectively. These findings indicated that *Zygosaccharomyces bailii* was present in both beverages but in smaller quantities, likely contributing to secondary fermentation processes. The mean log10_(Li, Wang et al.) values were determined as 4.60 for *Tella* and 4.48 for *Tej*, reflecting its limited but consistent presence.

Table 7: The mean counts of isolated yeast species from tej and tella in Raya kobo in 2025.

Isolated Yeast Species	Mean Maximum (cfu) – Tella	Mean Minimum (cfu) - Tella	Mean Maximum (cfu) - Tej	Mean Minimum (cfu) - Tej	Mean Log10 value	
					Tella	Tej
Saccharomyces cerevisiae	5.2×10^6	5×10^5	4×10^6	4×10^4	6.72	6.60
Zygosaccharomyces bailii	4×10^4	3.2×10^3	3×10^4	2×10^3	4.60	4.48
Hanseniaspora species	3×10^4	2×10^4	2×10^4	1.6×10^3	4.48	4.30
Pichia pastoris	3×10^4	3×10^2	3×10^4	2×10^3	4.48	4.30

The yeast *Hanseniaspora* species was found to be moderately present in both *Tella* and *Tej*. In *Tella*, the maximum mean count was recorded at 3×10^4 cfu, while the minimum count was 2×10^4 cfu. In *Tej*, a slightly lower count was observed, with a maximum of 2×10^4 cfu and a minimum of 1.6×10^3 cfu. These counts suggested that *Hanseniaspora* species played a role in the early stages of fermentation, but their presence was gradually reduced as fermentation progressed. The mean log10_(Li, Wang et al.)10 values were recorded as 4.48 for *Tella* and 4.30 for *Tej*, indicating a moderate contribution to the fermentation process.

The lowest yeast counts among the identified species were recorded for *Pichia pastoris*. In *Tella*, the maximum count reached 3×10^4 cfu, whereas the minimum count was found to be 3×10^2 cfu. Similarly, in *Tej*, the maximum and minimum mean counts were observed at 3×10^4 cfu and 2×10^3 cfu, respectively. These findings suggested that *Pichia pastoris* was present in both beverages but played a minor role in the overall fermentation process. The mean log10_(Li, Wang et al.)10 values were determined as 4.48 for *Tella* and 4.30 for *Tej*, confirming its lower presence compared to other yeast species.

The yeast count analysis revealed that *Saccharomyces cerevisiae* was the dominant species in both *Tella* and *Tej*, highlighting its primary role in ethanol production and fermentation. *Zygosaccharomyces bailii*, *Hanseniaspora* species, and *Pichia pastoris* were observed at lower counts, indicating their potential involvement in secondary fermentation or flavor development. The variations in yeast counts between *Tella* and *Tej* suggested that fermentation conditions, ingredient composition, and environmental factors might have influenced the microbial composition. These findings provided a detailed understanding of the yeast dynamics in Ethiopian traditional fermented beverages.

4.2. Discussion

4.2.1. Socio-demographic characteristics

The findings of this study reveal that all Tella and Tej vendors surveyed were female, indicating a gender-based dominance in the production and sale of these traditional beverages. This aligns with previous studies that have identified women as primary producers of home-brewed beverages in Ethiopia due to cultural and economic roles (Abegaz, 2007). The age distribution showed that the majority of respondents were above 44 years old (64%), suggesting that experience plays a significant role in beverage preparation. Similar trends have been reported in other studies, where traditional brewing is often conducted by older women who have acquired knowledge through generational transfer (Alemu et al., 2016).

Regarding education, a significant proportion of respondents (20%) were illiterate, while only 10% had attained secondary education or a diploma. This educational gap could influence the adoption of improved hygienic practices and business management strategies. Marital status analysis showed that a large proportion of respondents were widowed (56.7%), which may indicate their reliance on beverage preparation as a means of economic survival, a finding consistent with studies on women's economic activities in Ethiopia (Demeke, 2012). Furthermore, the religious homogeneity of the respondents, all identifying as Orthodox Christians, suggests that cultural and religious norms might influence their engagement in Tella and Tej production.

4.2.2. Knowledge of vendors on tej and tella preparation

The study revealed that all participants (100%) demonstrated a clear understanding of the traditional beverage preparation process, indicating that knowledge of fermentation techniques is deeply ingrained within the community. A significant majority (93.3%) reported preparing these beverages in their homes, highlighting the prevalence of household-based fermentation practices.

This widespread familiarity underscores the cultural significance of homemade fermented beverages, which have been passed down through generations and remain an essential aspect of local traditions (Feyissa et al., 2019).Furthermore, respondents reported employing specific hygienic measures during the preparation process, particularly the use of natural sanitizers such as Grawa (Vernonia amygdalina) and Weira (Cordia africana) to clean fermentation vessels.

These practices align with previous research findings, which emphasize the antimicrobial properties of these plant-based sanitizers in reducing microbial contamination and enhancing the efficiency of the fermentation process (Gizaw et al., 2018). The use of such traditional cleaning methods suggests that local knowledge contributes significantly to maintaining the safety and quality of the fermented beverages.

The socio-economic importance of Tella was unanimously recognized by all respondents, reflecting its multifaceted role in the community. It serves not only as a source of income for many households but also plays a crucial role in fostering social interactions and strengthening communal ties. Additionally, Tella is a key element in various cultural and religious ceremonies, further solidifying its importance in Ethiopian society. These findings are consistent with earlier studies that have highlighted the deep-rooted social and economic functions of fermented beverages in Ethiopia, emphasizing their role beyond mere consumption (Wolde et al., 2015).

The study also found that fermentation duration varied among respondents, with the majority (70%) reporting a fermentation period of seven days. This duration aligns with standard practices observed in traditional Tella preparation, which is known to yield an optimal balance of flavor and alcohol content. Previous research has similarly identified a one-week fermentation period as the ideal timeframe for achieving desirable sensory and chemical properties in the final product (Teshome et al., 2021). This consistency in fermentation duration among respondents suggests adherence to well-established preparation techniques that contribute to the beverage's characteristic taste and quality.

4.2.3. Physico-chemical parameters of tella and tej samples

The pH analysis revealed that Tej samples exhibited lower pH values, ranging from 3.63 to 3.94, compared to Tella, which had pH values between 4.49 and 4.94. This finding indicates that Tej is more acidic than Tella.

The increased acidity of Tej can be attributed to the fermentation process involving honey and Gesho (Rhamnus prinoides) leaves, which promote the growth of acid-producing microorganisms.This acidity plays a crucial role in enhancing the microbial stability of Tej, making it less susceptible to spoilage and improving its preservation.

These findings align with previous research by Alemayehu and Kebede (2019), which also reported a similar pH range and highlighted the importance of acidity in determining the quality and shelf life of Tej. Additionally, the analysis of Total Dissolved Solids (TDS) and Electrical Conductivity (EC) showed higher values in Tella compared to Tej, suggesting a denser composition. This difference can be attributed to the ingredients used in their production. Tella is typically brewed using grains such as barley and sorghum, which contribute to its higher solid content and, consequently, higher TDS and EC values. In contrast, Tej is primarily honey-based, resulting in lower TDS and EC levels. These findings are consistent with the study by Getachew et al. (2020), which demonstrated that grain-based fermentations generally exhibit greater solute concentration and electrical conductivity due to the presence of complex carbohydrates, proteins, and minerals. This difference in composition not only influences the physicochemical properties of the beverages but also affects their sensory characteristics, fermentation efficiency, and nutritional content.

4.2.4. Identification of yeasts based on morphological characteristics

The dominant yeast species identified in both beverages was Saccharomyces cerevisiae, a well-documented microorganism renowned for its pivotal role in alcoholic fermentation. This finding aligns with prior microbiological studies on Ethiopian traditional beverages, which consistently identified Saccharomyces cerevisiae as the primary fermenting yeast (Ayele et al., 2017).

Additionally, the presence of Zygosaccharomyces bailii in Tella and Hanseniaspora species in Tej highlights species-specific fermentation dynamics. Notably, Hanseniaspora is known to contribute to the distinct fruity aroma characteristic of Tej, underscoring its influence on the beverage’s sensory profile (Bekele et al., 2021). These findings collectively emphasize the diverse microbial contributions to the fermentation processes and unique qualities of these traditional beverages.

4.2.5. Biochemical characteristics of the isolated yeast species

The carbohydrate fermentation patterns observed demonstrate that Saccharomyces cerevisiae efficiently metabolizes glucose, sucrose, and maltose, solidifying its dominance in the fermentation process (Tafere et al., 2022). This metabolic efficiency is a key factor in its widespread use in alcoholic fermentation. Additionally, the variable fermentation of raffinose and trehalose among different yeast species highlights strain-specific metabolic differences, which may contribute to variations in the sensory properties of the final beverages. Furthermore, the high ethanol tolerance exhibited by both Saccharomyces cerevisiae and Zygosaccharomyces bailii underscores their critical roles in the fermentation of alcoholic beverages, as they can thrive in high-alcohol environments that inhibit other microorganisms (Mogessie, 2016). These findings collectively emphasize the importance of metabolic adaptability and ethanol tolerance in shaping the fermentation dynamics and quality of traditional beverages.

4.2.6. Mean CFU/ml of the isolated yeast species

The highest yeast counts were observed for Saccharomyces cerevisiae in both Tella (5.2×10^6 cfu) and Tej (4×10^6 cfu), confirming its predominant role as the primary fermenting agent in these beverages. This dominance is consistent with its well-documented efficiency in metabolizing sugars and producing ethanol. Lower counts of Zygosaccharomyces bailii, Hanseniaspora species, and Pichia pastoris suggest their secondary, yet significant, roles in contributing to flavor complexity and supporting fermentation progression. These non-dominant yeasts likely influence the sensory profiles of the beverages through the production of secondary metabolites. The variation in yeast counts between Tella and Tej can be attributed to differences in ingredient composition and fermentation conditions, which selectively favor the growth of specific microbial communities (Assefa et al., 2018). These findings highlight the interplay between microbial dynamics and fermentation parameters in shaping the characteristics of traditional fermented beverages.

5. CONCLUSIONAND RECOMMENDATIONS

5.1. Conclusion

The study provided comprehensive insights into the socio-demographic characteristics, knowledge, and practices of Tella and Tej vendors, as well as the physico-chemical and microbiological properties of these traditional Ethiopian beverages. The findings revealed that all vendors were female, with a majority above 44 years old, and varying educational backgrounds. Vendors demonstrated strong knowledge of the fermentation process, hygiene practices, and the socio-economic significance of these beverages.

The physico-chemical analysis highlighted that Tej exhibited higher acidity (lower pH) than Tella, contributing to its microbial stability. Tella, on the other hand, contained higher total dissolved solids (TDS) and electrical conductivity (EC), indicating a denser composition. Yeast identification confirmed that Saccharomyces cerevisiae was the dominant fermenting yeast in both beverages, while Zygosaccharomyces bailii, Hanseniaspora species, and Pichia pastoris played secondary roles, influencing fermentation and flavor development.

The microbial count analysis demonstrated the prevalence of Saccharomyces cerevisiae, with varying levels of other yeast species, reflecting differences in fermentation dynamics. The findings contribute valuable knowledge on the microbial composition and quality attributes of Tella and Tej, which can aid in optimizing fermentation practices and ensuring product safety.

5.2. Recommendations

Based on the findings of this research the following recommendations are suggestd:

- Conduct targeted training programs for Tella and Tej vendors to enhance their knowledge of proper fermentation techniques and hygiene practices.
- Reinforce proper cleaning techniques for fermentation containers, including the effective use of traditional cleaning agents such as Grawa and Weira.
- Establish regular monitoring and inspection of fermentation facilities to ensure compliance with sanitary standards.
- Standardize fermentation processes to maintain optimal pH levels, particularly in Tej, which exhibited higher acidity levels. This could enhance product consistency and microbial stability.
- Promote the controlled use of Saccharomyces cerevisiae as the dominant fermenting yeast to ensure efficient alcohol production and flavor development.
- Encourage the use of laboratory testing for physico-chemical parameters such as pH, TDS, and EC to ensure product safety and quality.
- Establish cooperative groups or associations to facilitate knowledge sharing, training, and access to better resources.
- Implement regulatory measures and periodic testing to ensure Tella and Tej remain safe for consumption.
- Recognize and respect cultural and religious aspects of Tella and Tej consumption, ensuring that interventions align with traditional practices while promoting safety and hygiene.

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Appendix

Woldia University

Post Graduate Studies

Department of Biology (Applied Microbiology)

Research questionnaires

Objectives of Questionnaires

Dear respondent, these questionnaires was designed for identification of yeast species in Tella and Tej fermentation in Kobo and Robit town, North Wollo zone, amhara region, Ethiopia.

Instruction:I.please give answers briefly and clearly. II. Circle a letter of your choice where there are alternatives.

I. Personal information

A. Sex: M__ F__

B. Occupation: student,_Government- worker___ maid___ Farmer ___

C. Education status; A. none B. 1-4 C. 5-8 D. 9-10 E. 11-12 F.

Diploma G. degree and above

D. Religion: A. Orthodox B. protestant C. Muslim

II. Knweledge of respondents about Tella fermentation

1. Do you know Tella? Yes or No

2. What are the materials (furniture) needed for the preparation of Tella? _____

3. What are raw materials (cereals) used for Tella

Preparation? _____

4. What are cereals for making malt and ways of malting for tella?_____

5. How would you keep the quality and safety of Tella ?

6. Duration of Tella process from preparation to consumption? A. 6 days B.7 days C,8 days

7. What is the potential of Tella spoilage after ready to consume? A, Spoiled after

1-2 days B. Spoiled after 3-6 days C. Spoiled after a week

8. What is the factor behind the spoilage?

A. lack of safety B. increasing amount of malt C. its limited shelf life

9. What is socioeconomic value of Tella fermentation?

A. family drinking B .for holidays C. for social works D. for weeding

ceremonies E .for source of income F. for all G .others (clarify)

12. How is the alcoholism of Tella ? A. Has no alcohol B. has low alcohol C. has high alcohol

*Note! If you have additional comment on Tella fermentation please write down.

III. Knweledge of respondents about Tej fermentation

1. What are raw materials (cereals) used for Tej fermentation?

_____

2. What are ways (procedures) for Tej preparation?

_____

3. What quality and hygiene is kept during Tej fermentation?

_____

4. How many days are required for Tej fermentation?(From preparation to Consumption)_____

5. How many days are required for Tej consumption after it is already started consuming?

A.1-2 days B. 3-5 days C. a week D. 2-4 weeks

6. Is it possible to make Tej with out using honey or sugar? ,,,,,,,

7. What is the socio economic value of Tej fermentation?

A. for social works B. for holidays C. For weeding ceremonies D. for source of income E. others

8. How is the alcoholism of Tej? A. Has no alcohol B. has low alcohol C. has high alcohol

Thank you for your cooperation

v

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES
DEPARTMENT OF BIOLOGY

ISOLATION AND CHARACTERIZATION OF DOMINANT YEAST SPECIES FROM TELLA AND TEJ IN RAYA KOBO, NORTH WOLLO, ETHIOPIA

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A Thesis submitted to the Graduate School of Woldia University in Partial Fulfillment for the award of Master of Science Degree in Applied Microbiology

MARCH, 2025

WOLDIA,ETHIOPIA