Frontiers in Environmental Microbiology
2025, Vol. 11, No. 2, pp. 52-78
https://doi.org/10.11648/j.fem.20251102.14

Research/Technical Note

Microbiological and Nutritional Analysis of Borde "*USHA*" *an* Ethiopian Traditional Fermented Beverage in Yem Zone Central Ethiopia

Addisu Shewaye Mengesha[1, 2, *]

[1]Institute of Health, Faculty of Public Health, Department of Environmental Health Sciences and Technology in Applied Ecology, Jimma University, Jimma, Ethiopia

[2]School of Public Health, College of Medicine and Health Sciences, Yem Saja Secondary High School, Saja, Ethiopia

Abstract

Usha is a lightly white and thick consistency a traditional cereal-based fermented beverage commonly prepared from a mixture of locally available cereals and low alcoholic beverage consumed locally by native people Yem zone of central Ethiopia. However, scientific findings on the beverage regarding its fermentation process, microbial quality, and physicochemical and nutritional analysis are studied. This study aimed to investigate the microbiological, physicochemical, and nutritional status of Usha. A cross-sectional and laboratory-based experimental study design was followed by socio-demographic information related to traditional preparation techniques and socio-economic significances of the product. Microbiological safety analytical techniques of the samples, microbial succession due to the course of controlled fermentation of Usha, nutritional dynamics for the isolation, characterization of the microorganisms, and physicochemical and nutritional parameters were evaluated under laboratory conditions following standard microbiological techniques Total of 30 Usha samples were collected from Yem zone. Data analyses were performed using the Statistical Package for Social Sciences, version 27. T vending sites market and (houses) at the study site. The results indicated that approximately 96.36% of the producers of Usha are young-aged women, with 79.09% engaged in farmer. Microbial analysis showed that yeasts dominated Usha with a mean count (Log CFU/mL) of 6.74±0.1, followed by lactic acid bacteria (6.54±0.1). A total of 250 bacteria and 150 yeast isolates were characterized and grouped into genera. Fortunately, Salmonella was not detected. The early fermentation time of Usha was dominated by aerobic mesophilic bacteria, Enterobacteriaceae, and Staphylococci, with the highest mean counts (Log CFU/ml) of 6.09 ±0.01, 3.88±0.01, and 4.2±0.01, respectively. At the end of fermentation, the lactic acid bacteria and yeasts had the highest mean counts (Log CFU/mL) of 7.6±0.04 and 7.5±0.09, respectively, with significant differences ($p>0.05$) in counts. During Usha fermentation, the pH dropped (4.09 ±0.01 to 3.41±0.06). The bacterial profiles of Usha samples were dominated by (mean log cfu/ml) Yeast (6.74±0.04) LAB (6.54±0.70). The Usha samples had mean values for pH (3.90±0.5), titratable acidity (0.5±0.11), alcohol content (3.54±0.01), moisture content (78.03±0.9), ash (2.74±0.3), fat (1.54±0.2), protein (15.99±1.5), carbohydrates (3.5±0.3) undesirable microbes exceeded the permitted limits, highlighting the need for improved hygiene practices and the use of starter cultures to enhance both the safety and overall quality of Usha fermentation Detection of some food borne pathogens in Usha samples calls for improvement of handling practice as the detected pathogens could be post production contaminants as the physicochemical properties of the ready to consume Usha are stringent for most microbe.

[*]Corresponding author: addisushewaye12@gmail.com (Addisu Shewaye Mengesha)

Received: 8 April 2025; **Accepted:** 23 April 2025; **Published:** 26 June 2025

Keywords

Fermented Beverage, Microbial Dynamics, Nutritional Analysis, Physicochemical Properties, Usha

1. Introduction

1.1. Background of Study

Fermentation has been used for thousands of years to produce various foods and beverages across the globe. Traditional fermented cereal beverages were essential components of many ancient civilizations and are usually known as beer; the recipes are passed down from generation to generation and are still popular in many developing nations [84]. These drinks are locally produced fermented products based on the skills of household occupants following traditional knowledge techniques [4]. These traditional methods have led to fermented beverages constituting 20%-40% of individual diets and accounting for approximately one-third of global food consumption [17].

In Africa, traditional fermented beverages (TFBs) perform an important function. Consumers tend to recognize these beverages as types of food rather than just beverages, and older rural women have indigenous knowledge about these beverages [12]. However, fermented beverages are still commonly produced on a small scale in many African countries [25], including Ethiopia [13].

Africa stands out as a continent with a rich variety of fermented foods, which are an integral part of the Ethiopian diet. Ethiopia is one of the countries, rich in cultural diversity and well-known for its diverse traditional fermented beverages being processed and consumed by various ethnic groups. people of Ethiopia produce various locally made fermented beverages by using a variety of basic ingredients, including barley, maize, sorghum, Teff, and barley malt [38].

Ethiopians prepare a wide range of traditional fermented beverages and drinks using cereals. Some well-known examples include *Tella, Borde, Shamita, Cheka, Korefe, Keribo,* and *Bukuri*. *Tella* is considered an alcoholic beverage, while the others are either low alcoholic or non-alcoholic. And [46].

Fermented products, made from both plant and animal sources, play a crucial role in the human diet because microorganisms cause physical, chemical, and nutritional changes in these products. In many parts of Ethiopia, villagers prepare fermented beverages using maize, sorghum, millet, barley, or a combination of these cereals. Food fermentation has been a vital part of human civilization for a long time, serving as a way to preserve food, improve taste, and enhance nutritional value. [35].

The physicochemical and nutritional composition of Ethiopian traditional fermented beverages is closely linked to their ingredient composition and the changes in these parameters during fermentation play a crucial role in shaping their microbial profiles. Among these beverages, Tej stands out for its remarkable alcohol content, recorded at 8.23 ± 2.07. On the other hand, Borde offers a different nutritional profile, characterized by significant levels of carbohydrates, proteins, and ash. Specifically, it contains 6.88 ± 1.74 g/100 ml of carbohydrates, 3.20 ± 0.39 g/100 ml of proteins, and 0.78 ± 0.12 g/100 ml of ash [59].

Fermentation is one of the oldest methods of food preservation and is deeply rooted in traditional cultures and village life. Even today, fermentation is widely practiced at the household or village level in many countries. However, industrial-scale fermentation operations are relatively rare [49].

Usha is a traditional fermented beverage that is widely consumed in the western central regions of Ethiopia, particularly by the Yem people. It has low alcohol content and is made through a fermentation process that lasts from two to three days, using mainly roasted barley malt flour, combination of different cereals teff; maize, millet, barley the quality and safety of *Usha* can vary depending on the raw materials, processing methods, and environmental conditions. *Usha* (one particular beverage) has not been documented as a traditional fermented beverage in Ethiopia. Consequently, no scientific studies have been conducted on its traditional production techniques, microbiology, physicochemical properties, and nutritional analysis. Therefore, the target of this study was to fill this gap by documenting the traditional preparation of *Usha* (an undocumented locally fermented beverage) and present study to assessing its microbiology, physicochemical characteristics, and nutritional, analysis. By adding on this unique aspect of Ethiopia's traditional fermented beverages.

Both adults and children in the Yem area of southern and central Ethiopia frequently consume *Usha*. It is short shelf life and is usually consumed during celebrations, holidays, and for household consumption This beverage holds great significance as it serves as an affordable substitute for meals and also an important cultural drink, offering a low-cost food option for low-income consumers.

1.2. Statement of the Problem

The quality and safety of fermented beverages widely used in industrial-scale fermentation, many traditional fermented beverages in Ethiopia However, there is a lack of scientific knowledge and understanding of the Ethiopian fermented beverages household processing techniques, associated microorganisms, nutritional value, safety, and potential to support the livelihood of the Ethiopian population [81].

Producing high-quality TFBs can provide significant benefits: they offer a source of income for producers and affordable options for consumers. Unfortunately, many Ethiopians are overlooking these beverages due to lifestyle changes and the availability of certain raw materials. This trend has resulted in less motivation among local brewers to continue producing traditional products, despite their strong cultural significance within the local community [60]. It is important to study the characteristics of various traditionally fermented beverages to understand how to produce microbial interaction, physicochemical parameters, and nutritional composition [70].

Especially *Usha*, a low-alcoholic drink consumed by the Yem people. It is one of particular beverage no scientific studies have been conducted on its traditional production techniques, microbiology, physicochemical properties, and nutritional composition *Usha* beverage traditionally fermented beverages is essential for understanding their production processes, microbial analysis, physicochemical parameters, and nutritional analysis [25]. This knowledge gap limits for improving the quality and safety of *Usha*, as well as its nutritional and cultural value. Therefore, the problem that will be addressed by this study is the microbial dynamics, physicochemical changes, and nutritional analysis of *Usha* involved in the fermentation process under laboratory condition, and the evaluation of their quality and safety of the final product. Traditional fermented Usha is produced at the household level in study area and the microbial, physico-chemical and nutritional analysis have not been investigated, documented as a TFB in Ethiopia & reported before.

1.3. Research Questions

1. What is the traditional practice of preparation technics of *Usha* in the study area?
2. What is the microbial load present in Usha samples?
3. Which microorganisms are dominant in the fermentation of Usha?
4. What is the nutritional analysis and physicochemical changes of *Usha* collected from the study are?
5. What are the microbial dynamics, physicochemical changes, and nutritional analysis of Usha prepared under laboratory conditions?

1.4. Objectives of the Study

1.4.1. General Objective

The general objective of this study is to assess the Microbiological and nutritional analysis of *Usha* an Ethiopian traditional fermented beverage in Yem Zone Central Ethiopia.

1.4.2. Specific Objectives

The specific objectives to be covered in this study are.

1. Document the traditional preparation of Usha in the study area.
2. To assess the microbial load of Usha.
3. To characterize the dominant microorganisms of Usha.
4. To determine the physicochemical and nutritional analysis of Usha collected from the study area.
5. To assess microbial Dynamics, physicochemical changes, and nutritional analysis of Usha prepared under laboratory conditions.

1.5. Significance of the Study

The significance of this study contributes to making people familiar with the microbial quality physicochemical properties, and nutritional quality as well as documenting *Usha* an Ethiopian traditional beverage that were used in a wide range of food marketing both in development and further study for industrialization, extension services to introduce some improved methods increase nutritional value of *Usha*, a traditional Ethiopian beverage. It will also evaluate the quality and safety of *Usha*, as well as its nutritional and cultural value. Furthermore, this study will contribute to the development of improved fermentation techniques and practices that can optimize the production and preservation of *Usha* and other similar fermented beverages. These advancements will benefit the local producers and consumers of *Usha*, especially low-income communities in the central regions of Ethiopia. Additionally, this study will open up new avenues for future research on the *Usha* preparation technique and other food and beverage industries, as well as the exploration of the potential health benefits for further extended research in this area.

2. Review of Related Literature

2.1. Fermentation

Fermentation is one of the oldest methods of food preservation and is embedded in traditional cultures and village life. Fermentation processes are believed to have been developed over the years by women to preserve food for times of scarcity, to impart desirable flavor to foods, and to reduce toxicity [70]. Today, fermentation is still widely practiced as a household or village-level technology in many countries, but comparatively, very few operations are carried out at an industrial level [32]. As a technology, food fermentation dates back at least 6000 years and probably originated from microbial interactions of an acceptable nature. Fermentation has enabled our ancestors in temperate and cooler regions to survive the winter season and those in the tropics to survive drought periods by improving the shelf life and safety of foods and beverages. The importance of fermentation in modern-day life is underlined by the wide spectrum of foods marketed both in developing and industrialized countries, not only for the benefit of preservation and safety but also for their highly appreciated sensory attributes. Fermented foods are treasured as major dietary constituents in numerous developing countries because of their keeping quality under ambient conditions - thereby contributing to food security - and because they add value, enhance nutritional quality and digestibility improve food safety, and are traditionally acceptable and accessible [70].

The fermentation process has been around for a long time and can be thought of as a biological way of food preservation that results in desired features such as good organoleptic properties in the final product [36]. It has gained popularity in many cultures where fermented foods are part of the daily human intake because of their nutritional benefits and variety of sensory qualities. Fermented foods and beverages are produced all over the world using diverse manufacturing techniques and raw materials [9] and work in the human body because of the presence of functional microorganisms and their ability to change the chemical components of raw materials [80].

Individuals with limited purchasing power have access to safe, inexpensive, and nutritious foods. Preservation and safeguarding of foods and beverages remain the principal objectives of fermentation, with wholesomeness, acceptability, and overall quality, having become increasingly valued features to consumers, especially in rural areas where old traditions and cultural beverages are long embedded in many cultures, and despite traditional production technologies remaining there is potential for extension services to introduce some improved methods, particularly those for hygiene and safety. However, it must be noted in this context, despite modern food biotechnology making significant technological advances, limitations in Infrastructure and existing low technologies in rural areas of most countries create challenges to keep abreast of global developments toward industrialization [32].

2.2. Diversity of Fermented Products in the World

Adversity of fermented products, including porridges and beverages (alcoholic and non-alcoholic) is produced from both edible and inedible raw [72]. These are well documented in a Food Administration Organization (FAO) publication series on fermented foods [25], and Fermented cereals and fermented roots and tubers are consumed as dietary staples throughout Africa, Asia, and Latin America, in various forms including bread, porridges, gruels, and pickles. A wide range of grains, fruit, and vegetables are also used to manufacture beverages, both thirst-quenching products (mostly non-alcoholic) and those that are generally alcoholic and consumed on special occasions, including festivals [33].

2.3. Importance of Socio-cultural and Economic Cereal Fermented Beverages

African cereal beverages are empirically derived from the spontaneous none or alcoholic fermentation of worth from germinated cereals (sorghum, maize, millet). They are produced and consumed in most parts of Africa where sorghum, maize, and millet grow. The preparation of many traditional fermented foods and beverages remains a household art. They are produced in homes, villages, and small-scale industries. Their manufacturing has its roots in African traditions, where they are highly influential in both sociocultural and economic spheres [18].

Indeed, they are often attached to the traditions of hospitality and friendliness and are part of the protocol of most families. They serve to seal harmonious relationships between individuals [58]. Today, African traditional beverage production has become a very important economic activity carried out by women. The sale of these products allows them to generate income for their families. Despite the technology that differs from one country to another and from one region to another, African traditional beverages have almost the same characteristics: a short shelf-life, none or low alcohol, a sour aspect, solids, and microorganisms in suspension, as well as taste and color characteristics with the low cost and the widespread availability in some populations [22].

2.4. Traditional Fermented Beverages in Ethiopia

Microorganisms of various groups appear to be involved in the fermentation of beverages indigenous to different parts of the world. The sources of microorganisms are usually the ingredients, and the traditional utensils used for fermentation processes. Initially, therefore, a wide variety of microorganisms are involved but most give way to more adaptive genera as the fermentation goes on. It may thus be said that different groups of microorganisms may undertake the initiation of fermentation of most traditional fermented beverages as far as sufficient fermentable sugars are available in the substrate. As fermentation proceeds, the environment becomes more and more acidic yeasts and lactic acid bacteria dominate the fermentation. These two groups of microorganisms usually determine the alcohol content and flavor of the final product [82].

2.4.1. Araki Fermentation

Araki is usually brewed in rural and semi-rural is a distilled beverage. It is a colorless, clear traditional alcoholic beverage that is distilled from fermentation products prepared in almost the same way as Tella except that the fermentation mass in this case is more concentrated [25]. Urban areas are used more commonly by farmers and semi-urban dwellers than by people who live in the cities. In cities, those who drink Araki are predominantly lower-class people or those who have become dependent on alcohol and cannot afford to buy industrially produced alcohol [84].

2.4.2. Cheka Fermentation

Cheka is a cereal and vegetable-based fermented beverage that is consumed in the Southwestern parts of Ethiopia, mainly in Dirashe and Konso. Consumed by all age groups, including infants, pregnant and lactating women drink Cheka. From observation an adult man on average drinks up to 8 liters of cheka per day [89].

2.4.3. Tej Fermentation

Tej is a home-processed fermented alcoholic beverage. It is also commercially available as honey wine. It is prepared from honey, sugar, water, and leaves of Gesho (Rhamnus peptides).

2.4.4. Keribo Fermentation

Keribo is an indigenous traditional, non-alcoholic, dark brown colored fermented beverage made and consumed in diverse parts of the country, including the Jimma zone, southwest Ethiopia with similarity to Boaz of Bulgaria, Albania, Turkey, and Romania. It is produced mainly from barley and sugar. Fermented Keribo constitutes a major part of the beverages being served on holidays, wedding ceremonies, and also as sources of income for many households in the Jimma zone. The popularity of this traditional fermented beverage is more reflected among religious groups and those who do not like alcoholic drinks. Being considered a non- or low-alcoholic beverage and it has poor keeping quality with a shelf-life of not more than a day or two it has a pronounced characteristic of deteriorating beverage at the end of 48 h of fermentation [12]. It is produced by an overnight fermentation of cereal (barley) predominantly by activities of LAB like the fermentation of Shamita [54].

2.4.5. Tella Fermentation

Tella is one of the Ethiopian traditional beverages, which is prepared from different ingredients. It is brewed from numerous grains and different cereals, which contain barley, corn, wheat, and sorghum, and also from teff and maize, although in some regions, millet and Rhamnus printed Pre-preparation steps were like that described [79]. The clay container (insert) will be washed with Grawa (Vernonia amygdalina) and water several times and, after that, smoked with wood from weyra (Olea European sub species. Cuspidate) to find a good fragrance or flavor and to be neat. During the preparation of the Tella, an average duration of six to eight days was required by the Tella producers during the winter season [11].

2.4.6. Borde Fermentation

Borde is a traditional fermented beverage made from maize, barley, or wheat and their malts. Its production is based on the natural fermentation of the ingredients. It is an opaque, effervescent, light brown beverage consumed while at an active stage of fermentation. It is a very popular meal replacement consumed by both children and adults in southern Ethiopia and some other parts of the country. Maize is the most common ingredient for the preparation of Borde. The malt is usually made of a mixture of cereals described in The processes of Borde preparation as practiced in southern Ethiopia. Cereal for malting is carefully cleaned, rinsed in water several times, and soaked in clean water until malting. The malt is then sun-dried, and a portion is milled into flour for immediate use. Equipment used for processing, such as clay pots, grinding stones, straw sieves, gourd bottles, etc., are locally available [12].

2.4.7. Shamita Fermentation

Is a widely consumed beverage in different regions of Ethiopia it has a thick consistency and is consumed as a meal replacement by most people who cannot afford a reasonable meal it is produced by fermenting roasted barley overnight Malt is not commonly used in Shamita fermentation although local Shamita brewers in Addis Ababa use it frequently, and starch is the only principal fermentable carbohydrate. The microorganisms responsible for fermentation are mostly from back-slopping using a small amount of Shamita from previous fermentation as well as from the ingredients and equipment. ready-to-consume Shamita has a high microbial count [106-107cfu(ml)-1], made up of mostly lactic bacteria and yeast. microorganisms make the product a good source of microbial protein. However, Shamita has poor keeping quality because of the high numbers of live microorganisms and becomes too sour about four hours after being ready for consumption [5].

2.4.8. Sensory Characteristics of Traditional Fermented Beverage

They are opaque containing suspended solids, with sweet-sour taste and odors or flavors characteristic pinkish-brown color, whitish grey to brown or light brown color, following raw materials. Moreover, alcoholic beverages are of effervescent aspect refreshing quality. During cereal fermentation, several organic acids and volatile compounds are formed, which contribute to a complex blend of flavors in the products [65].

2.5. Microbiological Quality

2.5.1. Aerobic Mesophilic Bacteria

Aerobic mesophilic Bacteria are significant in food microbiology as an indicator of microbiological quality as well as a measure of sanitation used during the preparation and handling of food and beverages. A higher load of bacteria spoils the food quickly and causes a loss of quality of food [37].

2.5.2. Staphylococcus Species

Staphylococcus aureus is gram-positive, cocci-shaped, non-motile, non-spore farmer, and facultative anaerobic. Catalase is mostly positive and contaminates food products during preparation and processing, it remains a major source of foodborne sickness. It is a normal flora of humans and animals that live in different parts of the body, such as in the nostrils, on the skin, and in the hair. *Staphylococcus aureus* causes skin and soft tissue infections such as abscesses, bloodstream infections, pneumonia, and bone [76].

2.5.3. Enterobacteriaceae

Enterobacteriaceae is a gram-negative, facultative anaerobic bacterium that includes several human pathogens (*Salmonella, E. coli, Shigella,* and *Yersinia*) and also a large number of spoilage organisms. These bacteria are widespread in soil, on plant surfaces, and in the digestive tracts of animals and are commonly isolated from foods and beverages. *Enterobacteriaceae* species are the highest probable number, proving clearly that poor hygiene meals could be sources of food-borne disease [50].

2.5.4. Bacillus Species

Bacillus is a gram-positive and aerobic spore-forming bacillus belonging to the genus Bacillus, and other related species play important roles in food poisoning and spoilage [66].

2.6. Health Benefits of Traditional Fermented Beverages

Many African-fermented beverages (AFB) consumed have therapeutic values. Drinks can have a direct positive impact on health through the interaction of consumed living microorganisms (such as yeast or bacteria) or indirectly through the intake of microbial metabolites created during the fermentation process [65].

Some studies have demonstrated that the beneficial bacteria contained in fermented foods support and help the digestive system assimilate food, providing for better nutrition and thus increasing the effectiveness of the immune system. Other studies have found that fermentation increases the acidity of beverages inhibits spoilage and can rid the food of poisonous bacteria.

2.6.1. Use as Probiotics

Immune Probiotics are usually defined as microbial food supplements with beneficial effects on consumers. Probiotics have great potential for improving nutrition, soothing intestinal disorders, improving the system, optimizing gut ecology, and promoting Overall health because of their ability to compete with sensory quality [86].

2.6.2. Improvement of Organoleptic Properties

Microbial fermentation makes the fermented beverage palatable and improves the organoleptic properties of texture, aroma, and flavor [90]. These organoleptic properties make fermented food more popular than unfermented one in terms of consumer acceptance [12].

2.6.3. Microorganisms Involved in Traditional Fermented Beverages

The fermentation of beverages native to different parts of the world appears to involve microorganisms from various groups such as substances, and traditional utensils used in fermentation operations are usually the origins of microorganisms [78]. In different studies on traditional fermented beverages, it appears that the predominant microorganisms in beverages are mainly lactic acid bacteria and yeast. Lactic acid bacteria in the mash before spontaneous fermentation consist mainly of *Lactobacillus, Leuconostoc, Pediococcus,* and *Enterococcus [23]*. The oldest and most economically relevant biotechnology is yeast's generation of alcoholic beverages from fermentable carbon sources that also 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 [86].

Yeasts play an important role in the creation of traditional African fermented foods and beverages particular, *Saccharomyces cerevisiae* has been called "mankind's most domesticated organism [51]. LAB is widely distributed in nature and able to promote fermentation by utilization of food nutrients and producing a variety of substances [64]. LAB is Generally Recognized as Safe (GRAS) and can be used well for medical and veterinary applications [33].

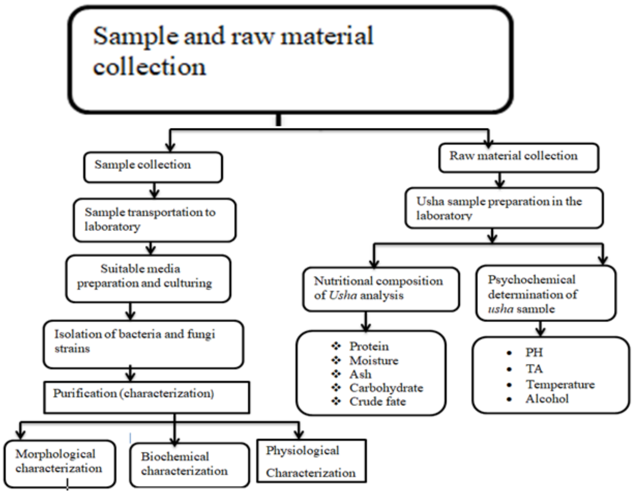

Figure 1. *Methodological and conceptual framework of the study.*

2.6.4. Preservative Properties

The preservative activity of LAB has been observed in some fermented products such as cereals. Lowering the pH to below 4 through acid production inhibits the growth of pathogenic microorganisms, which can cause food spoilage, food poisoning, and disease [52]. For example, LAB has antifungal activities. By doing this, the shelf life of fermented food is prolonged

3. Materials and Methods

3.1. Description of the Study Area

The studies were conducted in the Yem Zone. Yem zone is one of the central Ethiopia regional states located 240kms south of the capital city of the country (Addis Ababa). The Yem are an ethnic group living in south-central Ethiopia. Their native language is Yemsa, one of the Omotic languages. The neighbors of Yem include the Gurage, Hadya, and Kembata to the east across the Omo River and the Jimma (Oromia) to the south, north, and west. study will be conducted in Yem Zone located in the north-western apex of the Southern Nations, Nationalities and Peoples Regional State (SNNPR) of Ethiopia within coordinates of 7 °37'N to 8 °02' N and 37 °40' E to 37 °61' E. Yem occupies a surface area of 724.5 km 2 (IEP, 2014). The population size of fofa District is 52,490, of which 25,300 are males and 27,190 are females (Asnake, 2023). This district is known for agricultural production and cultivates a variety of cereals, including maize, sorghum, barley, wheat, and *teff*, as well as cash crops like ginger and coffee [8]. The cultural food and beverage Popular dishes include Kitfo (raw minced meat), and Doro Wot (spicy chicken stew), among others. Inset product like *Nattu*, *wotto, kocho (kobana)*, shenkallo (extract or grits of bean and pens) It serves as body building and give extra strength to the human being Additional cultural food like gonfo (porridge) servas meal breakfast on special occasions food given to the mother after child birth give extra strength during sickness nefro (boiled cereals and legumes) injera is also consumed by yem people as ceremonial food Beverage like *Usha* tella, arqe shameta and borde the district is partitioned into two agro-ecological zones; namely low land (*kola*) and midland (*Woina-dega*) of climatic conditions it contributing to the livelihoods of many families. Cultural Aspects: The kebeles often reflect the rich cultural heritage of the Yem people and other ethnic groups in the district, showcasing traditional practices, festivals, and local governance systems ‘ *Hebo,* the Yem New Year ritual, plays a vital role in resolving community disputes and conflicts Fofa District's kebeles play a crucial role in local governance, enhancing community involvement structure allows for localized decision-making that addresses specific community needs. And the implementation of government policies The Yem people possess extensive traditional knowledge of medicinal plants for treating various health issues. Traditional health practitioners among the Yem are skilled in using diverse plant species for health care. The cultural practices of the Yem regarding traditional food, beverage, and medicine could attract tourism interest.

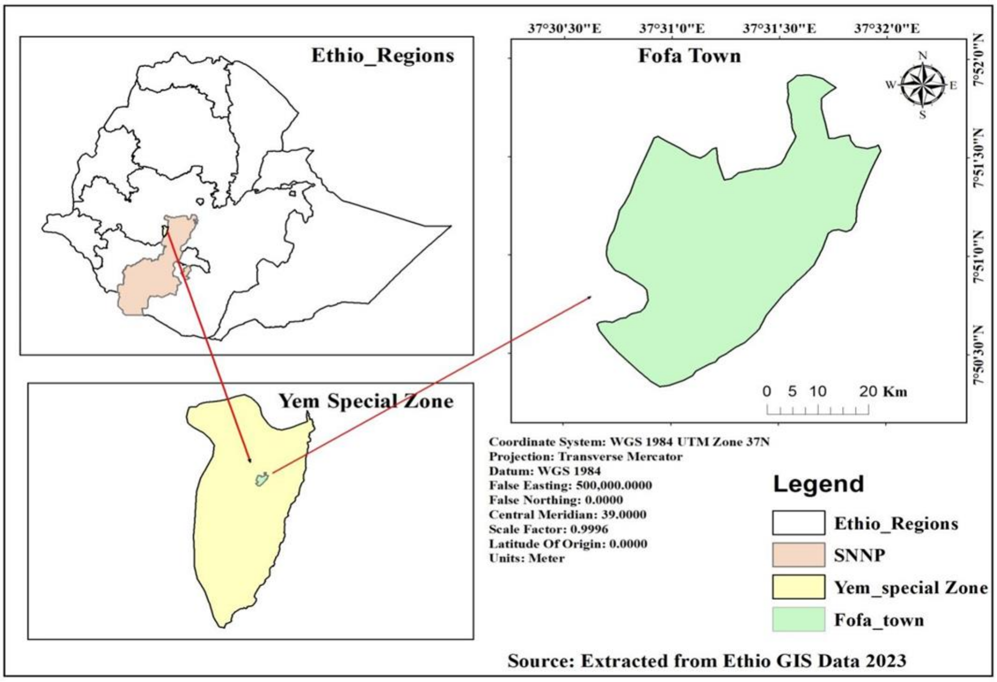

Figure 2. *Map of study area. Source: (https://openi.nlm.nih/detailedresul.php).*

3.2. Documentation of the Usha Preparation Technique

Traditional preparation techniques of Usha ingredients used, duration of fermentation, facilities of fermentation, and other related crucial information were gathered through a combination of on-site observations, interviews, and questionnaires. Interviews were performed in the respondents' native language, ensuring clear communication in both village and marketplace environments. This technique allowed for a comprehensive understanding of local practices and traditions. In addition, socio-demographic information and general details about the Usha were collected using questionnaires.

3.3. Study Design and Period

The study is designed to conduct experiments in the fermentation process of *Usha*, a traditional Ethiopian beverage A cross-sectional and experimental study design and laboratory-based experiment will be implemented from October 2016 to April 2017 to evaluate both *Usha* samples. And the laboratory fermented *Usha*.

3.4. Sampling Technique, Sample Size, and Data Collection

The present study investigates Usha, a TFB produced and consumed by the Yem community, particularly in the Yem Zone of central Ethiopia. The determination of sample size, sampling procedures, and data collection methods was done following the methodology shown by [41]. Accordingly, the district was purposively chosen due to its rich cultural heritage and extensive experience in the production and consumption of the Usha beverage. Of the 20 kebeles in the district, 10 were selected purposefully for the study based on the prominence of Usha production, as shown on the map (Figure 2). The study population consisted of respondents who were actively involved in the production, consumption, and sale of Usha during the data collection period from May 17-9, 2017 Individuals, who engaged in these activities outside of the specified data collection period, as well as respondents whose preparation techniques (ingredients and procedures) deviated from the traditional techniques were also excluded. A total of 110 respondents (study population) were selected for the study (10 participants each from the kebeles *of Kesheile, Toba, fofa, zemida, anger, oyia, Melaka, Ediya, semunema, deriy* and 11 participants from the kebeles of *duwagoner and nema*). Each selected kebele covered almost the same number because each area was identified based on 21 its greater engagement in Usha production. Data was obtained through interviews and questionnaires.

3.5. Sample Collection and Laboratory Scale Preparation

Locally prepared, 30 samples were collected randomly and based on the availability and willingness of the local producers to sell their beverage products (to give out the needed information) from the Yem zone. Three samples from each kebele) based on the availability of the product. The brewing households were selected based on their acknowledged experience with the drink and the readiness of local retailers to supply their products from each sample. About 500 ml of the *Usha* sample were collected in sterilized bottles and transported to Jimma University, Postgraduate Research Laboratory of the Department of Biology using an ice box. Samples were transported to the laboratory within 6 hrs. of collection and kept in a refrigerator at 4 ℃ until used for microbiological analysis and physicochemical and nutritional analysis.

The raw materials used for the lab-scale preparation of Usha were purchased from the open market in Jimma City (Merkato) and transported to Jimma University, Research and Postgraduate Laboratory. This was done to study microbial dynamics, nutritional analysis of selected food pathogens in the final products. The lab-scale preparation of Usha followed traditional techniques while avoiding cross-contamination.

3.6. Microbiological Analysis

Microbial enumeration was conducted following the methods of [61, 76]. Accordingly, 25 mL each of well-mixed samples were separately added to 225 mL sterile saline solution and homogenized in a flask at 100 rpm for 10 minutes in a homogenizer/shaker [19]. After homogenization, 1 mL of each sample was aseptically transferred into 9 mL of peptone water and mixed thoroughly using a vortex mixer. The homogenate was then serially diluted from 10^{-1} up to 10^{-7}, and the properly diluted samples were spread-plated in duplicate on pre-solidified sterile agar media (Oxoid). To enumerate distinct microbial populations, 0.1 mL aliquots of the diluted samples were spread plated onto various types of agar media. Plate Count Agar was specifically utilized for counting aerobic mesophilic bacteria (AMB) and aerobic spore-forming bacteria (ASFB), providing a general assessment of the microbial load. For the detection of Enterobacteriaceae, Violet Red Bile Glucose Agar (VRBGA) was employed. This medium is characterized by its ability to produce purple or pink colonies surrounded by purple halos, indicating the presence of these bacteria. Total coliforms were identified using Violet Red Bile Agar (VRBA), where characteristic purplish-red colonies with a reddish zone of precipitated bile suggested their presence.

To identify staphylococci, Mannitol Salt Agar (MSA) was utilized, with identification indicated by yellow colonies surrounded by red halos. For the enumeration of lactic acid bacteria (LAB), de Man, Rogosa, and Sharpe (MRS) agar was used, providing an optimal environment for their growth. Additionally, Potato Dextrose Agar (PDA) supplemented with 200 mg/L of Chloramphenicol was employed to count yeast and mold populations, effectively inhibiting bacterial growth while allowing fungi to develop. All media were incubated under aerobic conditions at

32 ℃ for 48 hours to facilitate bacterial growth. In contrast, yeast and mold counts were incubated at 28 ℃ for a period ranging from 2 to 5 days, allowing sufficient time for these fungi to proliferate. It is noteworthy that while all microbial groups were incubated aerobically, LAB required a different approach; these bacteria were cultivated anaerobically in an anaerobic jar (Hitech e– 601, China) to promote their growth under oxygen-free conditions.

3.7. Ethical Consideration

Approval for this study was secured from the Department of Biology at Jimma University, as well as from the Research and Ethical Review Board of the College of Natural Sciences. In addition, research permission (Letter of support) was granted by the officials at the study site. All data and sample collections were performed following the guidelines outlined in the permit.

4. Results

4.1. Socio-demographic Characteristics of Respondents in Study Area

A total of 110 respondents, 4 (3.6%) males and 106 (96.36%) females, responded to the questionnaire designed to study *Usha*, including its production techniques, raw materials used, and handling practices, among others. The respondents' ages ranged between 20-41 years A total of 32 *Usha* venders were engaged in Usha business and sold by women. All the vendors surveyed were female, with the most common age group being 31-35 years (54.5%). In terms of education level, 39.1% attended primary school, secondary school 10.9, and 1-4 (18.2)% while 31.8% were illiterate. The majority of the vendors were married (64.5%) and selling Usha.

Table 1. *Socio-Demographic Characteristics of study Population of Usha Consumers and Venders among Yem zone central Ethiopia.*

Characteristics	Alternatives	Frequency (n=110)	Percent
Gender	Male	4	3.6
	Female	106	96.36
Age	25-30	20	18.2
	26-31	14	12.7
	31-35	60	54.5
	36-41	16	14.5
Maternal states	married	71	64.5
	Divorced	27	24.5
	Single	12	10.9
Educational background	Illiterate	35	31.8
	1-4	20	18.2
	Primary. School	43	39.1
	Secondary School	12	10.9
Occupation	merchant	17	15.45
	Farmer	82	79.09
	Household	11	10
Religion	Orthodox	92	87.46
	Protestant	14	8.54
	Muslim	-	-
	Total	110	100%

4.2. Habits of *Usha* Consumption and Production in the Study

The findings provide important information among consumers:“ reasons of drinking of *Usha* were to quit thirsty (12.5%), for leisure (2.5%), to get happiness (6.25%), and to get energy or power (80%). According to the respondents“ Opinion, people usually drink usha infrequently (only some time) (81.25%) and the amounts Used at one go is two to four cup (tofu) (25-50%) most of the respondents have been drinking (1-6) year 87.5% Table 2.

Table 2. *Habits of Usha consumption in study area.*

Characters	Alternative	No of respondent	percentage%
Reason for drinking Usha	for thirsty	10	12.5%
	Give power	64	80%
	Make happy	5	6.25%
	As leisure	2	2.5%
Amount of Usha used to drink per day	4 cup (tofu)	40	50%
	3cup	35	43.75%
	2 cups	20	25%
How long have you been drinking Usha (years)?	1-6	70	87.5%
	7-10	31	28.18
	>10	9	11.25%
How often do you drink Usha?	Usually	30	37.5%
	Sometimes	65	81.25%
	In market day	15	18.75%

4.3. Knowledge and Practice of Usha Production and Consumption in the Study Area

All 100% of the 32 people who participated in this study were well experienced with Usha production and vending. The source of knowledge for Usha productions was 62.5% obtained from family while 37.5% obtained from relatives. All those who produce rely on the same type of raw materials for Usha production: maize (Zea mays), malted flour barley (Hordeum vulgare), teff, sorghum, and malt. Those Usha producers and vendors bought raw material from the market only, while 31.25% brought from both shop and market. Among the producers, (67.75%) produced Usha once a week and (9.37%) produced twice (15.62%) three times (68.75%) Types of water used to prepare Usha Tap water (78.12%) Spring water (15.62%) Place of Usha selling home (12.5%) and on market day (81.25%) Description of the Production Processes of Usha the basic processing steps of Usha fermentations are similar, although every Usha producer seems to have their own recipe based on traditional and the economic situation.

Table 3. *Knowledge of Usha production.*

Character	Alternative	No respondents	Percentage%
Usha producers	Male	4	12.5%
	Female	28	87.5%
Usha users	Male	50	62.5%
	Female	30	37.5%

Character	Alternative	No respondents	Percentage%
Familiar with Usha use and fermentation	Yes	110	100%
	No	-	-
Sources of knowledge for Usha production	Training	-	-
	From family	20	62.5%
	From relatives	12	37.5%
Types of ingredients used to prepare Usha	maize	32	100%
	Malt	32	100%
	Barley	32	100%
	Teff, millet	32	100%
Sources of raw materials to prepare Usha	Shop and market	22	68.75%
	Shop	10	31.25%
Types of water used to prepare Usha	Tap water	25	78.12
	Spring water	5	15.62%
Rounds of preparing Usha in a weak	Once	3	9.37%
	Twice	5	15.62%
	Three times	22	68.75%
Place of Usha selling	Home	4	12.5%
	Market	26	81.25%

4.4. Hygienic Practices During Usha Beverage Preparation and Consumption

All *Usha* venders have good cleaning habits of serving materials and service places, although they vary in frequency of cleaning as some clean only once (11.81%), twice (13.63%), or thrice (9.38%). All *Usha* producers and venders wear overcoats, while 28 (87.5) wear hair covers and only 4 (12.5%) of them do not. Furthermore, all producers have good habits of hand washing before and after the preparation of *Usha* and serving even though it was stored as it is for 24 to 48 hours. Most of them, 29 (85.3%), store *Usha* with all other home Materials and directly pitch from buckets by pitcher and dispense to (tofu) and vend for user while Only 26 (81.25%) of them use local shelves for temporary storage using pitcher and plastic Beaker (Table 4).

Table 4. *Hygienic practices during Usha preparation/vending and serving.*

Character	Alternative	No respondents	Percentage%
Cleaning habits of material and Selling place	Yes	32	29.09%
	No	-	-
Times of cleaning	once	13	11.81%
	Twice	15	13.63%
	Three times	3	9.38%
Reagents used to clean materials	Yes	27	84.37
	No	5	15.63
Types of reagents used to Water and	Ajax soap	29	90.62

Character	Alternative	No respondents	Percentage%
Omo Clean material Clean material	Water only	3	9.38
Habits of wearing hair cover	yes	28	87.5
	No	4	12.5
Habits of wearing over overcoat	yes	18	87.5
	No	-	-
Habits of hand washing	yes	32	100
	No	-	-
Storage condition of Usha	Refrigerator	-	-
	Shelves	6	18.75
	Bucket in Home room	26	81.25
Materials used to sell Usha	Pitcher	32	100%
	Cup (Tofu)	32	100%
	Plastic Beaker	32	100%

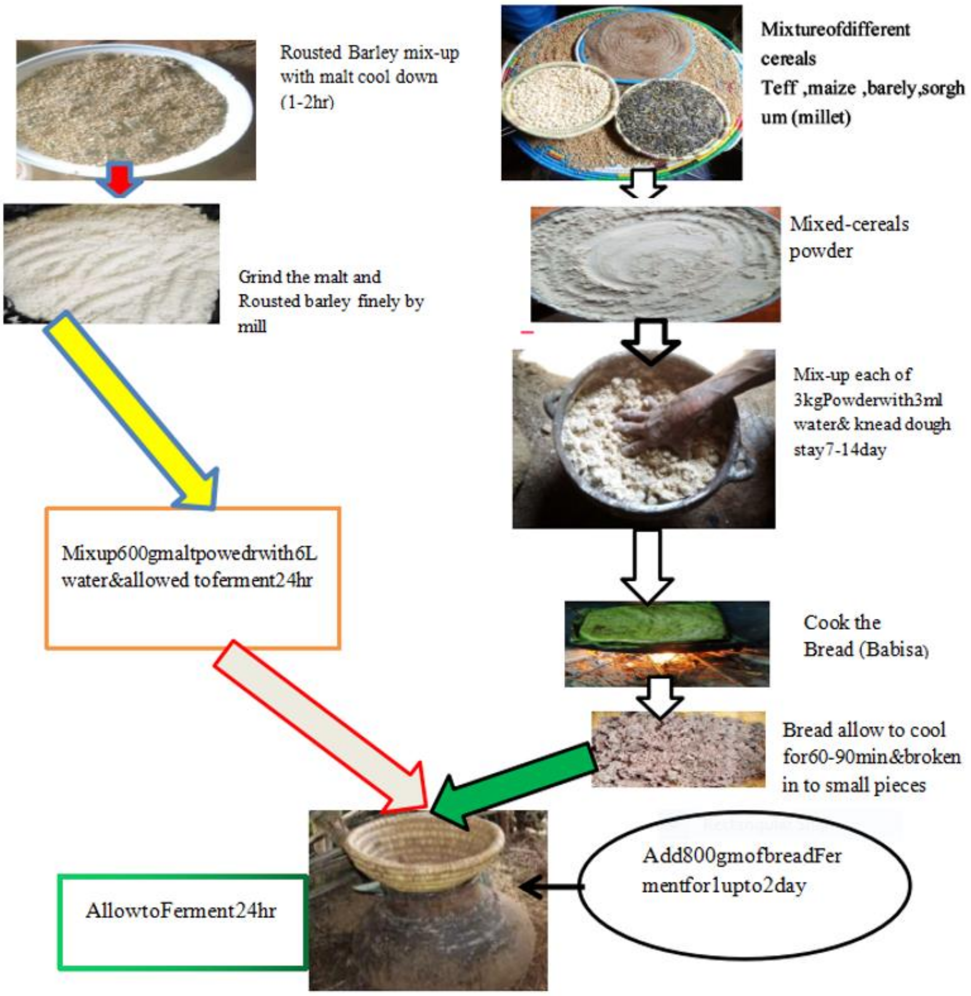

Figure 3. Traditional preparation techniques of Usha in the study area.

4.5. Description of the Production Processes of Usha

The basic Usha fermentation process steps are similar. Traditionally, Usha (Figure 2) was produced by using ingredients including barley (Hordeum vulgare), teff (Eragrostis teff), maize, sorghum, barley malt water, The cereals were ground and processed using locally available tools, such as clay pots (gawa), pans (mitad), plastic materials sieve and mills grand above cereals. The mean of ingredient proportions used in the preparation of Usha was 13.83 kg of mixture cereals from 6 to 30 kg, 600 kg of barley malt, ranging from 2 to 6 kg, 40 liters of water ranging from 10 to 70 liters water.

Figure 4. *Usha preparation under Laboratory condition.*

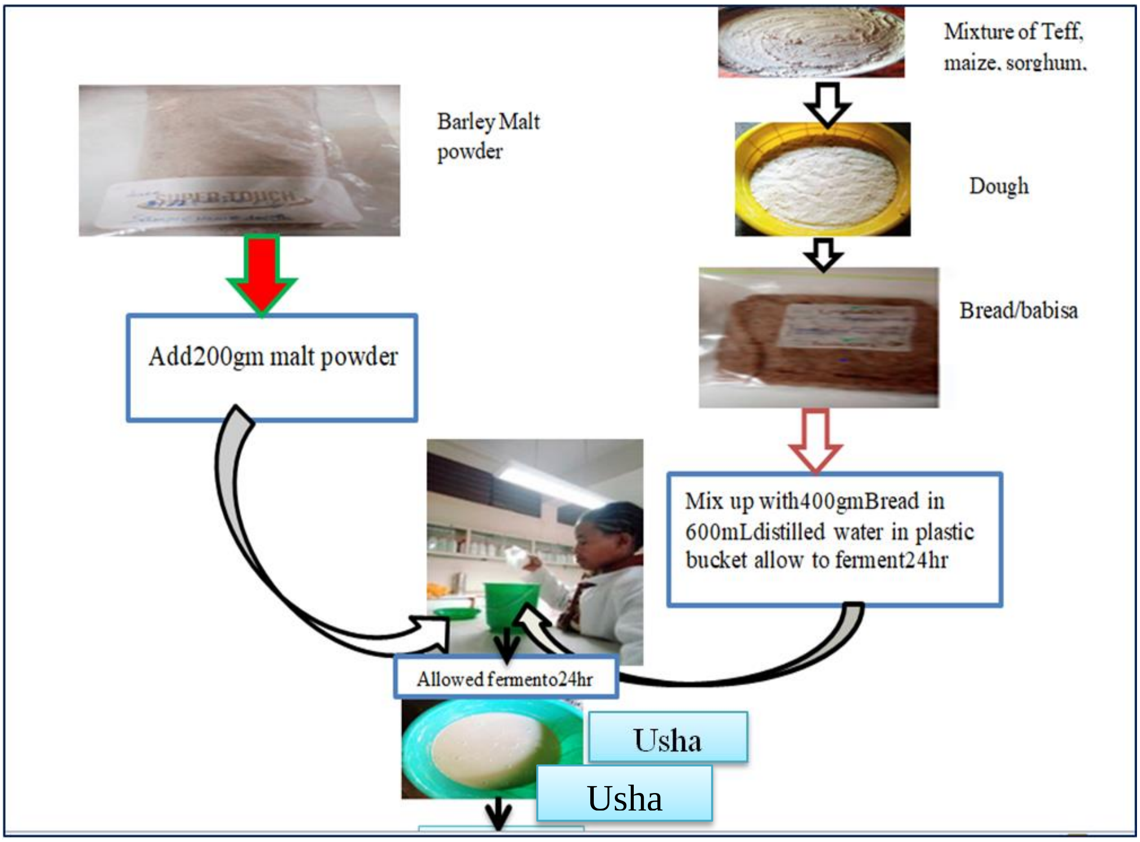

Figure 5. *Usha preparation under Laboratory condition.*

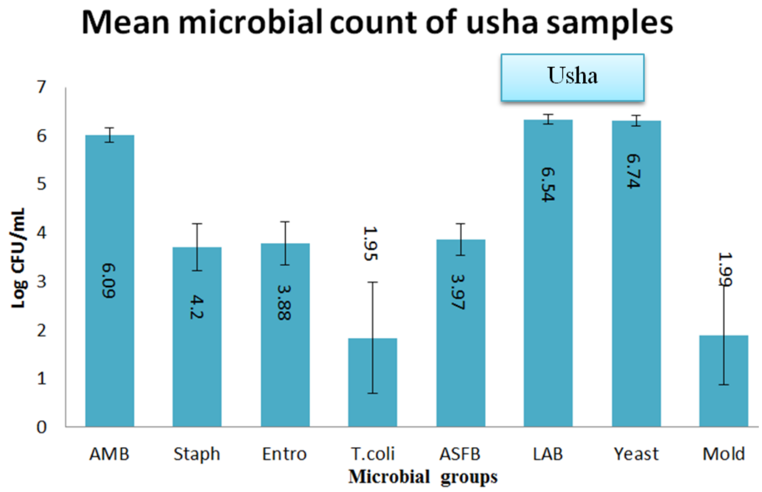

Figure 6. *Mean microbial count (log CFU/milliliter) of Usha sample collected from Yem zone central Ethiopia.*

4.5.1. Microbial Load of *Usha* Samples Collected From the Study Area

The results of microbial enumeration revealed that LAB and yeast were the dominant microbes in *Usha* samples and significantly outnumbered other genera, with AMB following closely behind. The mean counts of LAB, yeast, and AMB were 6.09 ± 0.1, 6.54 ± 0.1, and 6.74 ±0.14 Log CFU/mL, respectively, representing the highest average counts across all samples. Significant variations were observed among mean counts of LAB ($P < 0.05$), whereas no Significant differences were observed in the mean counts of AMB and yeast ($P > 0.05$). In contrast, the mean counts of *Enterobacteriaceae*, ASFB, and *Staphylococci* were nearly 4 Log CFU/mL, in nearly all *Usha* samples, with significant differences ($P < 0.05$) in mean counts between samples collected from different sites. Moreover, total counts of Coliforms and molds remained at below detectable levels (< 2 Log CFU/mL) across all samples.

4.5.2. Microbial Dynamics During lab-scale Usha Fermentation

At the initial phase of the Usha fermentation process (0 hrs.), Staphylococci, AMB and Enterobacteriaceae became dominant, with counts above ≥ 5 Log CFU/mL. AMB initially dominated at 6.09 ±0.01 then dropped for the first 6 h before experiencing an exponential decrease until 18 h. From 18- 24 h, AMB counts rebounded, then dropped again until 42 hours, before rising from 42 to 48 hours, after which there has been a steady decline. Mean counts of Staphylococcus, ASFB, Enterobacteriaceae, total coliforms, and molds displayed a similar manner as to how they change over time, although individual mean counts differed herein. Each group showed a decline from 0 to 18 hours, then an increase from 18 to 24 hours again from 42 to 48 hours, and finally, a downward trend towards the end of the fermentation process. Interestingly, the counts of Enterobacteriaceae, total coliforms, and molds dropped below detectable levels at the end of fermentation, yeast and LAB dominated. Yeast counts were below detectable levels until 6 hours but rose thereafter until the end of fermentation (72 hrs) and reached the maximum counts of 7.39±0.09 Log CFU/mL. LAB showed a steady increase for the first 18 hours, followed by exponential growth until the end of fermentation (72 hrs).

Table 5. *Physicochemical characteristics of Usha sample from Yem zone central Ethiopia.*

Physicochemical and nutritional	composition	No of the samples	mean ± SD	CV%
pH		30	3.90±0.5	12.5
Temperature (℃)			22.42±0.98	4.37
TA (%)			0.5±0.11	18.33
Moisture content (%)			78.03±0.9	1.74
Total solids (%)			19.05±0.7	3.5

Physicochemical and nutritional	composition	No of the samples	mean ± SD	CV%
Refractive index			1.4436 ±0.01	0.74
Specific gravity			1.0576 ±0.02	1.91
Alcohol (%)			3.54 ±0.21	5.72
Ash (%)			2.74 ±0.3	14.22
Total fat (%)			1.54 ±0.2	13.2
Titratable acidity (%)			0.6 ±0.3	11.3
Total protein (%)			15.99 ±1.6	10.72
Total carbohydrate (%)			3.5. ±0.3	13.45

Nutritional analysis of the beverage of Usha sample from the study area, the mean standard deviation protein content and carbohydrate, moisture content is high other are law nutritional content

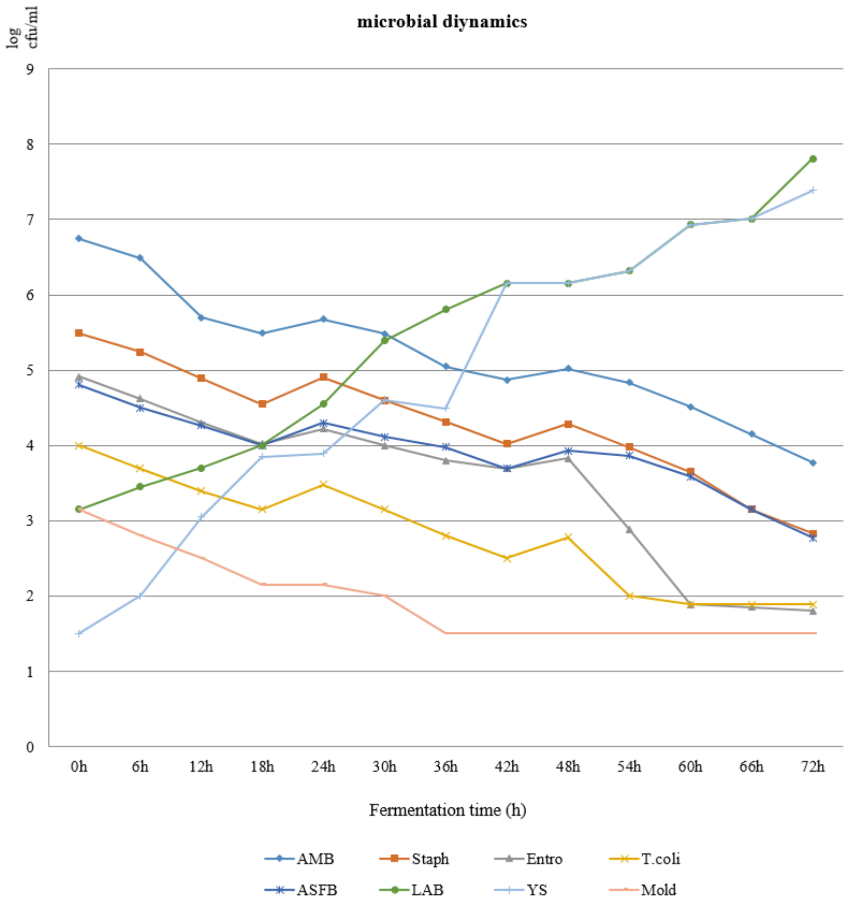

Figure 7. *Microbial dynamics (x ± SD) during fermentation of Usha beverages.*

Microbial dynamics (x ± SD) during fermentation of Usha beverages. Where: AMB=Aerobic Mesophilic Bacteria; Staph=Staphylococcus; T. coli=Total coliforms; ASFB=Aerobic Spore Forming Bacteria; LAB=Lactic Acid Bacteria

4.5.3. Characterization and Identification of LAB From Usha Samples

Among the fermented Usha samples, 154 LAB were characterized and grouped into genera from Usha samples, which consisted of laboratory-prepared samples and samples collected from the study area. Of these isolates, 105 (68.2%) were isolated from household vendors in the study area, whereas 49 (31.8%) were obtained through laboratory preparation of Usha; the classification of the isolates was based morphological, biochemical, and physiological characteristics. Morphological characteristics of the isolates showed a variety of shapes such as rod, cocci, or cocci in tetrads. Biochemical characterization shows that all isolates were Gram-positive, catalase-negative, spore-forming negative, and motility-negative. The result on the frequency distribution of LAB indicated that Lactobacillus 81 (51.9%) was the most prevalent genera followed by Enterococcus 37 (23.7%), Leuconostoc 24 (15.10%), and Pediococcus 11 (7.7%).

Table 6. Morphological, biochemical, and physiological characteristics of LAB genera.

Characteristics	**Group of LAB**			
I (n = 81)	***II (n = 24)***	***III (n = 37)***	***IV (n = 11)***	
Morphology	Rod	Cocci	Cocci	Cocci in tetrads
Gram reaction	+	+	+	+
Arrangement	Long/short chains	singly /pair	singly / pair	Tetrads
Catalase test	−	−	−	−
Motility test	−	−	−	_
Endospore test	−	−	−	_
CO_2 production from glucose	++	+ −	+/−	−
Carbohydrate				
Fermentation test				
Glucose	+	+	+	+
Lactose	+	+	+	+
Maltose	+	+	+	+
Mannitol	+	+	+	+
NaCl tolerance (%)				
2%	+	+	+	+
4%	+	+	+	+
8%	−	−	−	−
10%	−	−	−	−
Growth at				
Temperature (℃				
10	−	−	−	−
32	+	+	+	+
37	+	+	+	+
45	+/−	+/−	−	+
Tolerance to pH				
4.5	+	+	+	+-
9.6	+/−	+	_	_
Possible genera	Lactobacillus spp.	Leuconostoc sp.	Enterococcus spp	Pediococcus spp.

Key: −/+ = more than 80% did not grow, +/− = more than 80% grew, + = growth/or positive test
Result, − = no growth/or negative test result.

4.5.4. Frequency Distribution of Bacterial Genera in Usha Samples

A total of 250 bacteria isolates were analyzed from Usha samples, which included both household and laboratory-prepared samples. The isolates were characterized and grouped into their respective genera. Of the 250 bacterial isolates, 218 (87.2%) were Gram-positive, while 31 (12.4%) were Gram-negative. Among the 250 bacteria isolates identified in this study, the most prevalent genera were Lactobacillus 81 isolates (32.4%). Additionally, other genera detected included Enterococcus (37 isolates, 14.8%), Leuconostoc (24 isolates, 9.6%), Pediococcus (11 isolates, 4.4), Staphylococcus (35 isolates, 14%), and others.

Table 7. *Isolated microorganism in 30 samples of Usha locally collected from Yem zone central Ethiopia.*

S/N	microbial genera	Frequency	%of isolate
1	*Lactobacillus*	81	19.90%
2	*Pediococcus*	11	2.70%
3	*Leuconostocs*	24	5.89%
4	*Enterococcus*	37	9.09%
5	*Staphylococci*	35	8.6%
6	*Saccharomyces*	75	18.29%
7	*Streptococci*	16	3.90%
8	*Bacillus*	17	4.18%
9	*Mollerella*	10	2.43%
10	*Micrococcus.*	14	3.41%
11	*Citrobacter*	3	0.73%
12	*Pseudomonas*	18	4.42%
13	Alklagiese	1	0.24%
14	*Pichia*	14	3.43%
15	*Candida*	57	14%
16	*Proteus*	2	0.49%
17	*Klebsiella*	1	0.24%
18	*Providencia*	1	0.24%
Total		407	

Figure 7 frequency distribution of bacteria genera in the usha sample.

Identification and Characterization of LAB. A total of 154 LAB was isolated and analyzed from fermented Usha samples. The results of the biochemical characterization showed that all the isolates were Gram-positive, lacked catalase activity, were non-non-spore-forming, and were nonmotile. They were rods, cocci, or cocci in tetrads. The classification of the isolates was based on morphological, biochemical, and physiological characteristics C. Nikita and H. Desai, et al. (2012) The results indicated that 81 (52.59%) of the isolates belonged to the Lactobacillus genus, for pediococcus sp. 11 (7.14%) for Leuconostoc sp 24 (15.58%) and 37 (24.02)% for Enterococcus.

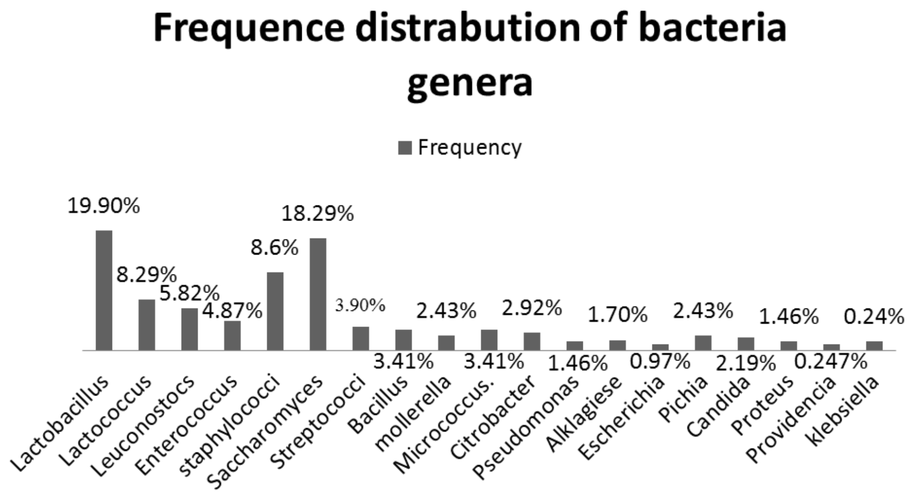

Figure 8. *Frequency distribution of bacteria genera in the usha sample.*

4.5.5. Characterization of Yeast

A total of 150 yeasts were isolated and characterized from Usha samples including those from laboratory-prepared samples as well as from household sample collections. Of these isolates, 95 (63.33%) isolates were obtained from household vendors, while 55 (36.66%) were derived from laboratory-prepared samples. Morphological characteristics of yeast isolates were determined by macroscopic and microscopic observation. The macroscopic analysis showed that the majority of yeast isolates exhibited a circular shape (89.4%), and small ones showed irregular shapes (11%). In terms of color, 35.63% of yeast was white, and 64.37% was creamy white. Microscopic analysis revealed a variety of shapes: oval (71.5%), ovoid (7.89%), lemon-shaped (5.65%), and circular (14.96%). The physiological characteristic test showed that 100% of the yeast isolates from Usha beverage tolerated a temperature at 37℃, salinity of 4 and 6%, and ethanol of 4%. On the other hand, only 1%, 0%, and 2.92% of yeast isolates tolerate 45℃, 15% salt, and 14% ethanol, respectively. In addition, approximately half of the isolates tolerated pH 2.

Yeasts' capacity to ferment sugars and generate CO_2 over time was assessed using the carbohydrate fermentation test yeasts; as a result, within 16 hours, 47 (31.3%) of the yeast isolates started generating CO_2 from glucose. Likewise, 17 isolates (10.7%) showed production of CO_2 from maltose during the same period. On the other hand, 23 yeast isolates (14.7%) fermented maltose without producing CO_2. Subsequent investigation showed that a sizable percentage of the isolates were unable to ferment specific sugars: 87 isolates (58%) were unable to ferment lactose, and 92 (61.33%) isolates were unable to ferment mannitol.

Table 8. *Some physicochemical and nutritional (x ±SD) analysis during laboratory-scale fermentation of Usha beverage.*

FT/h	PH%	T/A%	M /C%	TP/C%	Ash/C%	N/C%	CHO%	TF/C%	TS/C%
0hr	4.09±0.11^{a}	0.19±0.01^{f}	81.6±0.14^{g}	11.6±0.01^{a}	1.80±0.01^{g}	0.38±0.01^{fg}	17.32±0.014 ^{a}	1.06±0.01^{g}	32.45±0.01^{fr}
6hr	4.01±0.07a	0.20±0.01^{f}	83.40±0.14^{g}	11.45±0.01^{a}	1.81±0.01^{fg}	0.24±0.01^{fg}	16.15±0.01^{b}	1.27±0.01^{gf}	30.8±0.01^{fg}
18hr	3.94±0.11^{ab}	0.24±0.01^{ef}	84.67±0.14^{ef}	11.84±0.01^{b}	1.82±0.01^{efd}	0.20±0.01^{fg}	13.46±0.01^{c}	1.26±0.00^{f}	29.74±0.01^{ab}
24hr	3.81±0.02^{abc}	0.28±0.01$_{ef}$	85.88±0.14^{f}	12.37±0.01^{b}	1.83±0.01^{bcd}	0.40±0.01^{abc}	12.88±0.01^{d}	1.32±0.01^{gh}	28.16±0.14^{ab}
30hr	3.55±0.03^{abc}	0.30±0.01^{def}	86.71±0.28^{f}	13.89±0.01^{abc}	1.83±0.01^{bce}	0.76±0.01^{abc}	11.77±0.01^{e}	1.38±0.01^{ab}	25.85±0.01^{cb}
36hr	3.51±0.01^{abc}	0.36±0.01^{def}	87.89±0.1^{cd}	14.99±0.01^{abc}	1.85±0.01^{abc}	0.80±0.01^{bc}	11.2±0.01^{f}	1.42±0.01^{ab}	25.31±0.01^{cb}
42hr	3.49±0.14^{cde}	0.40±0.03^{cde}	88.56±0.14^{c}	14.34±0.1^{abcd}	1.86±0.01^{abc}	0.86±0.01^{bc}	10.63±0.01^{g}	1,53±0.01^{ab}	22.65±0.01^{cb}
48hr	3.40±0.07^{cde}	0.50±0.02^{cde}	89.89±0.28^{b}	15.98±0.1^{abcd}	1.87±0.01^{ab}	0.76±0.01^{b}	9.91±0.01^{h}	1.67±0.0^{abc}	21.46±0.01^{b}
54hr	3.58±0.02^{cde}	0.60±0.014^{ab}	90.63±0.01^{b}	16.34±0.01^{abc}	1.88±0.01^{ab}	0.75±0.14^{b}	8.8±0.01^{h}	1.7±0.01^{ab}	20.71±0.01^{b}
60hr	3.50±0.02^{de}	0.70±0.03^{abc}	91.34±0.01^{a}	16.78±0.041^{ab}	1.89±0.01^{b}	0.50±0.01^{a}	6.39±0.01^{i}	1.78±0.01^{a}	20.33±0.01^{a}

FT/h	PH%	T/A%	M /C%	TP/C%	Ash/C%	N/C%	CHO%	TF/C%	TS/C%
66hr	3.45 ± 0.01^{de}	0.80 ± 0.01^{ab}	92.56 ± 0.01^{a}	16.92 ± 0.041^{ab}	$1.90 \pm 0.01_{b}$	0.55 ± 0.01^{a}	2.3 ± 0.01^{j}	1.84 ± 0.00^{a}	19.13 ± 0.01^{a}
72hr	3.41 ± 0.01^{de}	0.89 ± 0.01^{a}	95.02 ± 0.01^{a}	17.08 ± 0.01^{a}	1.95 ± 0.00^{a}	0.66 ± 0.01^{a}	2.8 ± 014^{k}	1.91 ± 0.01^{a}	18.27 ± 0.1^{a}

The values represent mean ± SD, and the statistical analysis was determined by one-way ANOVA with Tukey post hoc test. Different letters in Superscripts along a column indicate a significant difference ($p < 0.05$), and the same letters indicate no significant difference ($p > 0.05$).
Key: Fermentation time; SD = Standard deviation; TA = Titratable acidity; MC = Moisture content; TSC= Total solid content; TFC=Total fat content; N/C=nitrogen content TPC=Total protein content, and CHO=Total carbohydrate content. The physicochemical and nutritional characteristics of the fermented beverage were assessed based on the beverage's fermentation times: 6-hour intervals

5. Discussion

Usha is a traditional law alcoholic whitish colored fermented beverage common in rural and urban areas of Yem zone central Ethiopia, some similarity with Tella, Teji, Areki and Korefe of Ethiopia [89]. The socio-demographic data reveals that a significant proportion of Usha vendors are women Aged 36-40, which aligns with the cultural perception of Usha preparation as a female-oriented task. This is comparable to the gender roles observed in Bukuri preparation in East Wollega, as noted by [13]. Usha beverages are used as a meal replacement by the majority of people who cannot afford to buy healthy and nutritious meals. Due to its widespread availability in the Yem Zone's open markets, this beverage has a significant socioeconomic impact on the neighborhood. [13] reported that this role was similar to that of Bukuri and Cabbage-shamata.

The majority of vendors only have primary education, which indicates that Usha vending may be a viable source of income for people with little formal education. Additionally, the fact that 64.5% of the vendors are married suggests that Usha Vending is a thriving business venture for people of all ages. Usha is a desirable option due to its low startup capital requirements.

During the study, from total of 110 people who participated in this study were familiar with Usha consumption. Sources of knowledge for Usha production: 32 women who were experienced in preparing *Usha* beverage fermentation was interviewed.

According to the knowledge and practices of respondents on the production and consumption of Usha, findings indicated that all the respondents (100%) were familiar with the Usha beverage, indicating its cultural significance and widespread acceptance within the community. The majority (62.5%) of the participants responded that sources of knowledge regarding Usha production were from families. This shows traditional knowledge transfer between intergenerational learning in maintaining cultural practices. The unanimous lack of special clothing usage among vendors, the surveys on Usha producers regarding hygiene practices and cleaning habits results showed that none of the respondents (100%) has been seen to wear coats while preparing Usha. This may raise questions about hygiene standards and customer perceptions in the food vending industry. Research has shown such studies by [80].

That advocating the wearing of protective clothing helps prevent the transfer of harmful germs from workers to food in service settings. Hence, the absence of such practices has been a key area for improvement as well as the need for better education concerning food safety standards. The socio-demographic characteristics of home venders of Usha in this study indicate that the majority of home producers of Usha were females, and the consumers are both males and females. This corresponds to previous studies regarding gender roles in Ethiopian food practices by [31] and also gender roles in Jikita preparation observed in West Shewa Zone, as reported by [39].

This may indicate that there are cultural expectations and practices in the study community where domestic and food-related roles are mainly delegated to women. The largest proportion of respondents (79.09%) was farmers, followed by merchants (15.45%), and housewives (10%). Almost half (87.46%) of the respondents were Orthodox Christian followers, while 8.54% were Protestant Christian followers. These reflect the cultural and spiritual contexts that influence social practices, including beverage consumption. The larger number of Christians in the study site is solely based on the wide settlement of followers of the specified religion in the study area.

According to local informants, the types of raw materials used to produce Usha were a Mixture of cereal maize sorghum, teff, barley, and malt powder predominantly sourced from the open market, reflecting the local agricultural produce and the vendors' reliance on market availability. It is produced 24 hours to 72 hours of fermentation.

As cited by [39] some of the ingredients utilized for fermented beverages such as barley, wheat, maize, and malted barley flour obtained from local markets indicate the diversity in agricultural products within the region and dependency of vendors on the market supply and Sources of water used to produce Usha were tap water 78.12%and spring water 15.62% and also, selling place of Usha were home and market 12.5%, 81.25% are respectively With mean counts of 6.54 ±0.1 and 6.74 ± 0.1 Log CFU/mL, respectively, LAB and yeast predominated in all 30 Usha samples taken from the households, according to the study's findings on microbial load analysis. Their ability to adapt to fermenting sugars in low-oxygen environments, outcompete other microbes, and produce products that prevent spoiling organisms may be the reason for their dominance.

According to [21], LAB and yeast have also been observed to predominate in the Tej samples. Additionally, [58] noted that yeast and LAB were important in improving the fermentation process and were dominant in the fermentation processes of Grawa, Tej, and Borde beverages. This state is essential for enhancing taste and consistency. This condition is crucial to improve flavor, texture, and even the preservation of several types of beverages [25]. The synergistic relationship between LAB and yeast plays an important role in the safety of fermented foods and beverages [25].

In the current study, the average counts of AMB Usha samples were 6.09 Log CFU/mL, which aligns with previous findings by [39] on Jikita samples taken from lactating mothers' homes, with an AMB mean count of 6.13 Log CFU/mL. The mean counts of AMB in Usha were higher than in the Shamita samples reported by [21] in their study.

The mean counts of AMB were higher than the standard limit (5 Log10 CFU /mL) recommended by the New South Wales Food Authority (NSW, 2009). AMB could be an indicator of quality rather than a food safety indicator because it doesn't give any direct indication about food safety but still helps in providing insight into the remaining shelf life of food products [20]. Moreover, the average counts for Enterobacteriaceae, ASFB, and Staphylococcus were recorded at 3.88, 3.97, and 4.2 Log CFU/mL, respectively, which was found at the at borderline of the standard level of the guideline [57]. This finding doesn't agree with those observed in Borde fermentation by [29]. In ready-to-eat foods, but high count of the Staphylococcus presence of these microbes is used as an indication of either post-processing contamination or inadequate cooking and post-fermentation handling [57]. Furthermore, adding water to the product could induce more chances of contamination. As emphasized by [81], a large portion of ready-to-eat foods within Ethiopia are contaminated by Enterobacteriaceae due to poor handling practices during transport, selling, and handling. Increase the microbial load, as they harbor ASFB, but have also been mentioned as possible contaminant sources for fermented beverages according to [2]. High counts of Aerobic Mesophilic Bacteria, Enterobacteriaceae, and Staphylococcus due to production and handling contamination. The occurrence of Enterobacteriaceae and Staphylococcus are evidence of poor hygienic conditions of Usha samples. During filtration time by hand contamination and skin these organisms may be contaminants of unsafe water used either to dilute the ready-to-use Usha or wash utensils. The utensils used for the preparation of Usha and serving are made low low-quality plastics bucket storing condition and necked cups that are difficult to clean [4].

However, the average counts for total coliforms and molds were less than the detectable level (< 2 Log CFU/mL) and considered acceptable according to the NSW (2009) guideline. This finding agrees with the work of [58], who recorded similar microbial reductions during Grawa, tej, and Borde fermentation.

Their findings have demonstrated the key role of fermentation dynamics as a critical process that regulates the unwanted microorganisms in local beverages to make them safe for consumption. The low undetectable levels of total coliforms and molds (< 2 log CFU/mL) in the Usha beverage might be due to several factors, including the acidification of the beverage during fermentation, which makes the environment unsuitable for such microbes. Besides, lactic acid bacteria and yeasts probably competed in the growth of coliforms and molds The result on morphological and biochemical characteristics of the LAB contributed to Usha fermentations showed that all the isolates 154 (100%) were Gram-positive, non-motile, and non-catalase-producing. Of all these isolates 154 (100%) could grow at both 32 ℃ and 37 ℃, while none could tolerate 10 ℃. This agrees with the previous report by Kitessa et al. (2022b) on the study of Shameta samples. The LAB genera that identified from Usha belonged to Lactobacillus, Leuconostoc, Pediococcus, and Enterococcus, supporting the findings of Djè et al. (2009), who studied mash before spontaneous fermentation. Out of a total of 154 LAB isolates, the genus Lactobacillus was the most dominant among the isolates with 81 (52.59%), followed by Enterococcus with 37 (24.02%). This is in agreement with a previous study by [26], who obtained a similar distribution and observed that Lactobacillus comprised 52% of the dominant genera in azo samples. The genus Lactobacillus is famous for containing numerous GRAS species, with several strains being important in food microbiology and commonly used as probiotics [43].

In the current study, out of a total of 407 microbial genera identified from Usha yeast genera such as Saccharomyces 75 (18.24%) and Candida 57 (14%) dominated the fermentation of Usha beverages. Similarly, a study by [41] identified 18 different yeast species from Borde samples collected in East Shoa and Jimma, with Saccharomyces cerevisiae being the most abundant (32%), followed by Wickerhamomyces anomalus (24%). This finding aligns with Bahiru et al. (2006), who reported the dominance of Saccharomyces cerevisiae (25%) in tej fermentation, followed by Kluyveromyces bulgaricus (16%). The variation in dominant yeast species across different fermentation processes may be attributed to differences in the sensitivity of methods used to characterize the isolates. Overall, studies on Ethiopian traditional fermented beverages (TFBs) consistently highlight the prevalence of Lactobacillus spp., Saccharomyces, and Candida spp. as the main contributors to fermentation [3].

The analysis shows that Staphylococcus aureus was present in 10 out of 30 samples (32.57%). However, none of the Usha samples from the study area contained Salmonella spp. This result supports the need for more research into the intrinsic parameters of the products, as reported by [21], who found S. aureus in Shameta samples. Similarly, 13 out of 30 Jikita samples (43.33%) had S. aureus, while no Salmonella spp. was found, according to [59]. S. aureus is most likely present in these drinks as a result of contamination during the fermentation process and at other points in time, including from raw ingredients. According to [83], S. aureus is a frequent contaminant in Ethiopian ready-to-eat foods, regularly Resulting from food producers' and vendors' improper hygienic practices. Acinetobacter 5 (1.22%), Enterobacter 2 (0.49%), Alcaligenes 1 (0.24%), Proteus 2 (0.49), Providencia 1 (0.24%), Pseudomonas 18 (4.42%), Bacillus 17 (4.18%), Staphylococcus 35 (8.6%), and Micrococcus 19 (4.7%) comprises the total of the 407 microbial isolates found from Usha samples in this study. Similarly, [59] identified these genres from Jikita samples. These bacterial genera are a sign of inadequate handling procedures from a safety point of view. Additionally, according to the FAO/WHO expert meetings on microbiological risk assessment, their abundance is not advised for the consumption of fermented beverages [42]. The high prevalence of Staphylococcus in humans may be related to the bacteria's widespread on human skin and nasal cavities.

The other microbes could also be attributed to the vendor's poor handling practice of ingredients and equipment during Usha preparation, these microbes could be found on cereals and water and joined to products during preparation.

Microbial dynamics of Usha Fermentation. In the laboratory, the microbial growth dynamics of the Usha fermentation process were studied at 6-hour intervals over 72 hours. The tests were performed at different times during the fermentation, such as 0 up, upto 72 h. The results showed that as the fermentation time increased, the number of LAB and yeast increased while the number of Enterobacteriaceae and coliforms and other potential pathogen decreased therefore, fermentation has a positive impact on the growth of beneficial microorganisms while simultaneously decreasing the number of pathogenic microorganisms the early-stage fermentation was dominated by microbial counts (>5 log CFU/mL) of AMB, Enterobacteriaceae, ASFB, and Staphylococcus. However, LAB and yeast were dominant at the end of fermentation, with maximum counts of 7.6±0.78 and 7.5±0.78, respectively. This observation aligns with the findings by [59] regarding Jikita microbial dynamics. Yeast and LAB dominated other microorganisms at the final stages of Usha fermentation due to their adaptability to acidic environments. Moreover, the counts of AMB, Enterobacteriaceae, ASFB, Staphylococcus, Coliforms, and molds exhibited an increase at 24 and 48 hours, followed by a decline. These findings demonstrate the significant role that the duration of fermentation plays in microbial growth and product formation.

For microbial identification of other all were different biochemical tests used and the used biochemical tests were:- Gram stain, Endospore test, Catalase test, motility test, Methyl Red (MR) test, indole test, urease test, TSIA test, SIM test, and carbohydrate fermentation test were based on the references as cited in [40, 85] and Bergey" s Manual of Systematic Bacteriology, John manuals of bacterial identification and [74], Lactic acid bacteria microbiological and functional aspects were used as reference). A total of 407 microbial isolates were characterized and identified to genus level from thirty Usha samples. Morphological, physiological, and biochemical characteristics of the isolated genus were grouped into respective genera. The cell morphology of all isolates was evaluated through microscopic observation variety of shapes: oval (71.5%), ovoid (7.89%), lemon-shaped (5.65%), and circular (14.96%). According to [89].

The physicochemical properties and proximate composition of Usha collected from the study area showed an average pH of 3.90, titratable acidity (TA) of 0.5%, alcohol content of 3.54%, temperature of 22.42 ℃, total solids of 19.05%, moisture content of 78.03%, fat content of 1.54%, protein content of 15.99%, carbohydrates of 3.5%, The pH, titratable acidity (TA), and alcohol content of Usha were found to be within the acceptable range, as per the standards [88]. These standards recommend optimal levels of pH (4.0-5.0), TA (0.16-0.8%), and alcohol content (2-8%) for ready-to-eat fermented beverages, confirming the quality and safety of the product. These findings reflect a higher pH, lower TA, and lower ethanol content compared to Tej samples reported by [20], who found a pH of 3.51, TA of 0.79%, and ethanol content of 11.04%. The total ash, protein, and fat content of Usha were found to be higher than the values reported by [39] for the Borde, Grawa, and Tej samples.

The physicochemical and nutritional composition of laboratory-prepared Usha revealed significant changes ($p < 0.05$) in their physicochemical and proximate properties from the beginning to the end of fermentation. The variation in all parameters of the samples might be linked to Differences in fermentation duration, ingredient types and composition, and microbial dynamics throughout the fermentation process. The finding revealed that the mean values of pH, total solid content, total and carbohydrates decreased from 4.09 to 3.41 32.45% to 18.27%, 17.32% to 2.8%, and, respectively, throughout the Usha fermentation process. A similar trend was observed in the study by [59], where the pH of Jikita prepared under laboratory fermentation decreased from 4.15 ± 0.08 to 3.77 ± 0.02. The reduction in pH during Usha fermentation is likely due to the metabolic activity of lactic acid bacteria (LAB) and yeast, leading to the production of organic acids such as lactic acid and acetic acid. This accumulation of organic acids contributes to a decrease in pH and an increase in TA. The decline in total carbohydrate content might be attributed to the breakdown of sugars into ethanol and CO_2 during fermentation. [2] highlighted that soluble sugar serves as a primary substrate for LAB during fermentation. Consequently, the natural fermentation of grains results in the depletion of available sugars, leading to a reduction in overall carbohydrate content.

These changes could underline the dynamic biochemical transformations occurring in Usha during fermentation. On the other hand, the mean values for titratable acidity (TA), moisture content, ash, total protein, and total fat increased from 0.19% to 0.89%, 81.6.% to 95.2%, 1.80% to 19.5%, 11.6% to 17.08%, and 1.06% to 1.91%, respectively, as fermentation progressed. These findings are consistent with those of Berhanu et al. (2023), who reported similar increases in these parameters during the maturation of Tej samples. The observed increase in moisture content might be attributed to the addition of water to the substrate before fermentation, as noted by [20].

The consumption of dry matter and the formation of water during both aerobic and anaerobic metabolic processes carried out by yeasts and lactic acid bacteria further explain the slow increase in moisture during fermentation [36]. The ingredients used, the amount of water added, and the length of fermentation could all affect the moisture content variation [21].

The observable rise in the amount of crude protein. Differences in the kinds and amounts of ingredients used, as well as the particular fermentation conditions, may be the cause of the variation in protein levels in Usha According to [20], fermentation commonly leads to an increase in in the amount of free amino acids, microbial metabolites, and total nitrogen content. Microbes create protein during fermentation.

The fat content of lab-prepared Usha may also be increased by using mixtures of different ingredients during the production process. Additionally, microorganisms produce enzymes that break down fats (lipids) into their constituent fatty acids and glycerol. During fermentation, these fatty acids build up and may be a factor in the apparent rise in fat content. Some microbial strains may also generate bioactive substances, such as fatty acids, which could increase the amount of fat in the fermented Usha beverage.

6. Conclusion and Recommendation

6.1. Conclusion

This study assessed the nutritional, physico-chemical, and microbiological analyses performed during a traditional preparation of Usha Yem Zone. For the first time, physico-chemical parameters, microbiology, fermentation processes, and nutritional analysis of Usha were examined. It emphasizes how women have traditionally produced Usha. The results demonstrate that the two most prevalent microbial groups in each sample were yeast and LAB. When Enterobacteriaceae are found at borderline safety levels, it indicates that the product was handled poorly and may have been contaminated after production. To stop microbiological growth during storage and consumption, this requires increased consumer awareness.

The lab-scale fermentation of Usha is started by the Enterobacteriaceae, AMB, Staphylococci, coliforms, and ASFB. But as fermentation developed and the nutritional and physicochemical characteristics shifted, LAB and yeasts turned into became dominant These microbes exhibited strong fermentative capacity and were least affected by environmental changes, offering potential for industrial applications and health benefits by inhibiting harmful microbes The study also demonstrated that, with the exception of pH, total solids, and carbohydrates, the physicochemical and nutritional characteristics of laboratory-prepared Usha improved with fermentation time. Better hygiene practices are necessary because the presence of Enterobacteriaceae in Usha suggests a risk of fecal pathogen transmission. Lactobacillus was the main force behind Usha fermentation, followed by yeasts and Enterococcus (among the LAB). Since Usha is a nutrient-dense meal replacement and beverage, production must be expanded on a large scale for the benefit of the Usha community. The findings presented here may be used as a basis for understanding the nutritional, physicochemical, and microbiological aspects of Usha production to increase process efficiency. The study's findings also affirmed the cultural and economic significance of the community.

Evaluating the Usha fermentation process and potential innovations, sociodemographic respondents can provide valuable information about the preservation of cultural heritage and the development of economic opportunities association within a grope to traditional fermented beverage products. Traditional fermented beverages are important both culturally and economically in specific regions of the Yem Zone in central Ethiopia.

6.2. Recommendation

Based on the Findings of this Study, the Following Key Recommendations are Proposed.

1. Usha beverages are growing rapidly due to increasing consumer demand for a healthy and sustainable product that was necessary.
2. The isolation of Enterobacteriaceae in ready-to-use Usha good indicator of producing and handling contamination. Therefore, producers' discussions should be on personal hygiene and how to improve Usha quality and safety were necessary.
3. It is mandated the use of protective clothing during *Usha* production to safeguard both the product and the producers.
4. From a nutritional perspective, consuming *Usha* beverages with 12 to 48 hours of fermentation is better. Because, beyond this time, the carbohydrate composition decreases, resulting in a drop in energy.
5. To encourage Usha documentation and preservation of traditional fermentation techniques and nutritional availability to maintain cultural heritage and promote sustainable beverage production practices where needed to Yem zone central Ethiopia.
6. The dissemination of the research findings and recommendations to the relevant stakeholders and the public

was completed.

7. To encourage further investigations into optimizing production techniques, physicochemical and nutritional Characteristics, minerals, vitamins, and bioactive compounds and enhancing the quality of traditional fermented Usha beverage.

Abbreviations

ANOVA	Analysis of Variance
ASFB	Aerobic Spore-forming Bacteria
AOAC	Association of Official Analytical Chemists
CFU	Colony-forming Unit
E. coli	*Escherichia Coli*
GRAS	Generally Recognized as Safe
H_2O_2	Hydrogen Peroxide
KOH	Potassium Hydroxide
LAB	Lactic acid Bacteria
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
SPSS	Statistical Package for the Social Sciences
TFB	Traditional Fermented Beverage
YPDE	Yeast Peptone Dextrose Extract

Acknowledgments

First of all, I want to thank my Almighty GOD for his permission of my living for in this world full of health. I would like to express my sincere appreciation to my advisor Professor Kitessa Hundera, for his additional constructive comments and suggestions to the accomplishment of thesis work. I would also like to thank Jimma University for admitting me to the program, and Yem Zone Educational Office for their sponsorship, and the Yem Zone for allowing salary payment to follow me. I want to thank the Saja Administration Office for all the necessary cooperation from woreda down to each kebele during the research undertaking. I am forthrightly thankful to the Department of Biology for permitting me to use the laboratory, chemicals, media, and equipment, in addition to facilitating the research work I want to thanks Jimma University College of Agriculture and Veterinary Medicine (Department of nutritional sciences); College of Natural Sciences for technical supports and laboratory facilities Next to this I Want to thank my family for supporting me for their immeasurable supports and contribution, especially my father Shewaye Mengesha, for her every constructive idea which was basic from my very beginning up to know and also, my wife Amarech Fajii my love and gratitude to my beloved my Wife ‘who is always in my mind for his love, understanding, and encouragement throughout my stud.

Declaration

I declare that this thesis entitled "Microbiological and nutritional analysis of Borde <<Usha >> Traditional Fermented Beverage in YEM zone, central Ethiopia", submitted for the Award of Degree of Doctor of Philosophy in applied ecology to Jimma University, is my original work. The content of this thesis is based on the experiments that I have performed myself. This thesis has not been submitted for any degree to other University.

Author Contributions

Authors were involved in analyzing and interpreting the data, drafting and revising the manuscript as well as approving the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

References

[1] Achi, O. K. (2005). The potential for upgrading traditional fermented foods through biotechnology. *African Journal of Biotechnology*, *4*(5), 375-380.

[2] Adelekan, A. O., Alamu, A. E., Arisa, N. U., Adebayo, Y. O., & Dosa, A. S. (2013). Nutritional, microbiological and sensory characteristics of malted soy-kunu zaki: an improved traditional beverage.

[3] Abafida, R. (2013) Morphology of karibo fermentation: an Ethiopian traditional fermented beverage,” Pakistan Journal of Biological Science, 16: 1113-1121.

[4] Abawari, R. A. (2013). Microbiology of keribo fermentation: an Ethiopian traditional fermented beverage. *Pakistan Journal of Biological Sciences: PJBS*, 16(20): 1113-1121.

[5] Abdoul-Latif, F. M., & Bassolé, I. H., & Dicko, M. H. (2013). Proximate composition of traditional local sorghum beer “dolo” manufactured in Ouagadougou. *African Journal of Biotechnology*, 12(13): 1517-1522.

[6] Abegaz, K. Beyene. F, Langsrud. T, and Narvhus, J. A. (2002). Parameters of processing and microbial changes during fermentation of boride, a traditional Ethiopian beverage. *Africa Journal of Food Technology*, 7: 85-92.

[7] Agbagwa, O. Rani S. and Halami P., (2017). Antibacterial potential components of Bacillusspecies and antibiotics residues in branded and unbranded honey samples from Nigeria. Afr. J. Biotechnol, 16(2): 58-64.

[8] Agize, M., Demissew, S., & Asfaw, Z. (2013). Indigenous Knowledge On Management Of Home Gardens And Plants In Loma And Gena Bosa Districts (Weredas) Of Dawro Zone, Southern Ethiopia: Plant Biodiversity Conservation, Sustainable Utilization And Environmental Protection. *International Journal of Sciences: Basic and Applied* Research (IJSBAR), 10: 63-99.

[9] Asnake, A. (2023). *Farm land management practices among smallholder farmers: The case of Tarcha Zuria Woreda, Dawuro Zone, Southwest Ethiopia Reginal State*, M.Sc Thesis, Hawassa University, Ethiopia.

[10] Auesukaree, C. (2017). Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation. *Journal of bioscience and bioengineering*, 124(2): 133-142.

[11] Aruwa, C. and Olatope, S., O, A. (2015). Characterization of Bacillus Species from Convenience Foods with Conventional and API Kit Method: A Comparative Analysis. Journal of Applied Life Sciences International. 3(1): 42-48.

[12] Bell, V., Guina, J., & Fernandes, T. H. (2023). African fermented foods and beverages. potential impact on health. *Microbial Fermentations in Nature and as Designed Processes*, 293-322. 93- 322. https://doi.org/10.1002/9781119850007.ch12

[13] Chali, B., Dabassa, A., & Bacha, K. (2024). The art of production and microbial dynamics of Bukuri and Cabbage-Shameta: A traditional fermented beverage of Ethiopia. *International Journal of Gastronomy and Food Science*, 35, 100850.

[14] Chay, C., Dizon, E. I., Elegado, F. B., Norng, C., Hurtada, W. A. and Raymundo, L. C. (2017) Isolation and identification of mold and yeast in medombae, a rice wine starter culture from Kompong Cham Province, Cambodia. Food Research 1 (6): 213 - 220.

[15] Cheesbrough, M. (2006). District laboratory practice in tropical countries. Cambridge University Press, New York, USA.

[16] Chelule, P. K., Mbongwa, H. P., Carries, S., & Gqaleni, N. (2010a). Lactic acid fermentation improves the quality of amahewu, a traditional South African maize-based porridge. *Food Chemistry*, 122(3): 656-661.

[17] Chileshe, J., van Den Heuvel, J., Handema, R., Zwaan, B. J., Talsma, E. F., & Schoustra, S. (2020b). Nutritional composition and microbial communities of two non-alcoholic traditional fermented beverages from Zambia: A study of mabisi and munkoyo. Nutrients, 12(6), 1628. https://doi.org/10.3390/nu12061628

[18] Chelule, P. K., Mokoena, M. P., & Gqaleni, N. (2010b). Advantages of traditional lactic acid bacteria fermentation of food in Africa. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 2: 1160-1167.

[19] C. O. Chukuezi, “Food safety and hyienic practices of street food vendors in Owerri, Nigeria,” Studies in Sociology of Science, vol. 1, no. 1, pp. 50-57, 2010.

[20] Das, G., Patra, J. K., Singdevsachan, S. K., Gouda, S., & Shin, H. S. (2016). Diversity of traditional and fermented foods of the Seven Sister states of India and their nutritional and nutraceutical potential: a review. *Frontiers in Life Science*, 9(4): 292-312.

[21] D. A. Kitessa, K. Bacha, Y. B. Tola, M. Murimi, E. Smith, and S. Gershe, “Nutritional compositions and bioactive compounds of ‘Shameta’, a traditional home made fermented porridge provided exclusively to lactating mothers in the western part of Ethiopia,” Heliyon, vol. 8, no. 2, article e08990, 2022.

[22] De Lemps, A. H. (2001). *Boissons et civilisations en Afrique*. Presses Univ de Bordeaux. 67-191.

[23] Djè, M.., Aka, S., Nanga, Y, Z., Yao, K, C, and Loukou, Y, G (2009). Predominant lactic acid bacteria are involved in the spontaneous fermentation step of tchapalo process, a traditional sorghum beer of Côte d’Ivoire. Res. *Journal of Biology Science*. 4(7): 789- 795.

[24] Djè, M. K., N’Guessan, K. F., Djeni, T. N. D., & Dadie, T. A. (2008). Biochemical changes during alcoholic fermentation in the production of “tchapalo”, a traditional sorghum beer. *International Journal of Food Engineering*, 4.

[25] Fadahunsi, I. F., Ogunbanwo, S. T., & Fawole, A. O. (2013). Microbiological and nutritional assessment of burukutu and pito (indigenously fermented alcoholic beverages in West Africa) during storage. *Nature and Science*, 11(4): 98-103.

[26] Getahun, B., & Bobe, B. (2015). Impacts of Land Use Types on Selected Soil Physico-Chemical Properties of Loma Woreda, Dawuro Zone, Southern Ethiopia. *Science, Technology and Arts Research Journal*, 4(4): 40-48.

[27] Ginjo, G., & Abiyot, L. (2019). Determinants of farmers' decision to use improved land management practice in Gindara watershed, southern Ethiopia. *Ethiopian Journal of Environment and Development*. 2(2): 17-34.

[28] Gram. C. (1884). Ueber die isolirte Färbung der Schizomyceten in Schnitt-und Trocken präparaten. Fortschritte der Medcin. National Institute of Allergy and Infectious Diseases. (2): 185-189.

[29] Gizaw. B. (2018). Potential microbial ecology in Ethiopia and ex-situ conservation effort. Annals of Microbiology and Infectious Diseases, 1: 08-39.

[30] Gregerson, G. (1978). A rapid method for the distinction of gram-negative from gram-positive bacteria. *European Journal of Applied Microbiology*. 5: 123-127.

[31] Greppi, A., Directed, L. C., & Jespersen, L. (2014). Yeasts for nutritional improvement of traditional african fermented products. *Università degli studi di Torino*. PhD thesis.

[32] Holzapfel, W. H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology*, 75(3): 197-212.

[33] Hoque, M, F., Akter, K. Hossain, M. Rahman, M., Billah, and Islam, K. 2010. Isolation, Identification, and Analysis of probiotic properties of Lactobacillus spp. from selective regional yogurts. *World Journal of Dairy and Food Science*. 5(1): 39-46.

[34] Hossain, N., Zaini, J. H., & Mahlia, T. M. I. (2017). A review of bioethanol production from plant-based waste biomass by yeast fermentation. *International Journal of Technology*.

[35] Hotessa, N., & Robe, J. (2020). Ethiopian indigenous traditional fermented beverage: the role of the microorganisms toward nutritional and safety value of fermented beverage. *International Journal of Microbiology*, 2020: 10.

[36] Hunduma, T. (2013). Research trends in modern food fermentation biotechnology and Ethiopian indigenous traditional fermented foods and beverages research achievements: a review. *Journal of Science and Sustainable Development*, 1(2): 71-86.

[37] Jacob, R. (2010). Microbial Quality of Ready to Eat Foods Available to populations of different Demographics. Submitted to Drexel University. The USA.

[38] Jans, C., Meile, L., Kaindi, D. W. M., Kogi-Makau, W., Lamuka, P., Renault, P., Kreikemeyer, B., Lacroix, C., Hattendorf, J., Zinsstag, J., Schelling, E., Fokou, G., & Bonfoh, B. (2017). African fermented dairy products. *International Journal of Food Microbiology*, 250: 27-36.

[39] Kalui, C. M., Mathara, J. M., & Kutima, P. M. (2010). Probiotic potential of spontaneously fermented cereal based foods - A review. *African Journal of Biotechnology*, 9(17): 2490-2498.

[40] Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial cell factories*, 17(1): 1-21.

[41] Kurtzman, C. P., Fell, J. W., & Boekhout, T. (2011). Definition, classification, and nomenclature of the yeasts. In The Yeasts (pp. 3-5). Elsevier.

[42] Laetitia, M. M., Joseph, H. D., Joseph, D., & Christian, M. (2005). Physical, chemical and microbiological changes during natural fermentation of "gowé", a sprouted or non sprouted sorghum beverage from West-Africa. *African Journal of Biotechnology*, 4(6): 487-496.

[43] Lautié, E., Rozet, E., Hubert, P., Vandelaer, N., Billard, F., Zum Felde, T., Grüneberg, W. J., & Quetin-Leclercq, J. (2013). Fast method for the simultaneous quantification of toxic polyphenols applied to the selection of genotypes of yam bean (Pachyrhizus sp.) seeds. *Talanta*, 117: 94-101.

[44] Lee, M., Regu, M., & Seleshe, S. (2015). Uniqueness of Ethiopian traditional alcoholic beverage of plant origin, tella. *Journal of Ethnic Foods*, 2(3): 110-114.

[45] Lei, V., Friis, H., & Michaelsen, K. F. (2006). Spontaneously fermented millet product as a natural probiotic treatment for diarrhea in young children: An intervention study in Northern Ghana. *International Journal of Food Microbiology*, 110(3): 246-253.

[46] Lemi, B, W., 2020 "Microbiology of traditionally fermented beverage and condiments," *International Journal of Microbiology*. https://doi.org/10.1155/2020/8891259

[47] Kanghae, M., Eungwanichayapant, N., and Chukeatirote. (2016). Characterization of *Bacillus Species* Exhibiting Strong Proteolytic Activity Isolated from Thua Nao Acta Alimentaria. 45 (1): 11-19.

[48] Marshall, E., & Mejia, D. (2011). *Traditional fermented food and beverages for improved livelihoods, FAO Diversification booklet* (Issue 21).

[49] Mezgebe, A. G. (2018). *Sorghum waxy and high protein digestibility traits and their relationship with malting and dough-based product making quality* (Doctoral dissertation, University of Pretoria).

[50] Mogessie, A. (2006). A review on the microbiology of indigenous fermented foods and beverages of Ethiopia. *Ethiopian Journal of Biological Sciences*, 5, 189-245. https://doi.org/10.4314/ejbs.v5i2.39036

[51] Mogmenga, L., Marius, K. Somda, Lewis, I. Ezeogu, Jerry, Ugwuanyi, and Alfred, S. Traoré (2019) Yeasts biotechnologies application and they are involved in African traditional fermented foods and beverages. *International Journal of Advanced Research.* 7(2): 44-62.

[52] Moonga, H. B., Schoustra, S. E., Linnemann, A. R., van den Heuvel, J., Shindano, J., & Smid, E. J. (2021). Influence of fermentation temperature on microbial community composition and physicochemical properties of mabisi, a traditionally fermented milk. *LWT*, *136*, 110350.

[53] Muchtaridi, M., Ida, M., Nunu, H., & Wiwiek, I. (2012). Determination of alcohol contents of fermented black tape ketan based on different fermentation times using specific gravity, refractive index, and gc-ms methods. Journal of Microbiology, Biotechnology and Food Sciences, 2, 933-946.

[54] Mulaw, G., & Tesfaye, A. (2017). Technology and microbiology of traditionally fermented food and beverage products of Ethiopia. *African Journal of Microbiology Research*, 11(21): 825-844.

[55] Muyanja, C. M. B. K., Narvhus, J. A., Treimo, J., & Langsrud, T. (2003). Isolation, characterisation and identification of lactic acid bacteria from bushera: A Ugandan traditional fermented beverage. *International Journal of Food Microbiology*, 80(3): 201-210.

[56] N'Guessan, F. K., N'Dri, D. Y., Camara, F., & Djè, M. K. (2010). *Saccharomyces cerevisiae* and *Candida tropicalis* as starter cultures for the alcoholic fermentation of tchapalo, a traditional sorghum beer. *World Journal of Microbiology and Biotechnology*, 26(4): 693-699.

[57] Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science and Nutrition*, 6(8): 2446-2458.

[58] Nkwe, D. O., Taylor, J. E., & Siame, B. A. (2005). Fungi, aflatoxins, fumonisin B1 and zearalenone contaminating sorghum-based traditional malt, wort and beer in Botswana. *Mycopathologia*, 160(2): 177-186.

[59] Nyanzi, R., & Jooste, P. J. (2012). Cereal-Based Functional Foods. *Probiotics*, 8: 161-197.

[60] Nielsen, M. (2018). Handwerkliche Braukunst und innovative Technologie: der kommunikative Spagat zwischen Tradition und moderner Technik. https://doi.org/10.1007/978-3-658-21537-8_16

[61] Ogodo, A., Ugbogu, O., & Ekeleme, U. (2015). Bacteriological quality of commercially prepared fermented Ogi (Akamu) sold in some parts of South Eastern Nigeria. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 9, 677-680.

[62] Ogunbanwo, S. T., Adewara, A. O., & Fowoyo, P. T. (2013). Effect of fermentation by pure cultures of Lactobacillus fermentum I and Saccharomyces cerevisiae as starter cultures in the production of burukutu. *New York Science Journal*, 6(1): 73-81.

[63] Ohlemiller, K. K., McFadden, S. L., Ding, D. L., Lear, P. M., & Ho, Y. S. (2000). Targeted mutation of the gene for cellular glutathione peroxidase (Gpx1) increases noise-induced hearing loss in mice. Journal of the Association for Research in Otolaryngology, 1, 243-254.

[64] O'Shea, E. F., P. D. Cotter, C. Stanton, R. P. Ross, and Hill, C. 2011. Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: Bacteriocins and conjugated linoleic acid. International Journal of Food Microbiolog 152(3): 189-205.

[65] Oyewole, O. A., & Isah, P. (2012). Locally fermented foods in Nigeria and their significance to national economy: a review 1: 92-102.

[66] Ozturk, G., & Young, G. M. (2017). Food evolution: the impact of society and science on the fermentation of cocoa beans. *Comprehensive reviews in food science and food safety*, 16(3): 431-455.

[67] Phiri, S., Schoustra, S. E., van den Heuvel, J., Smid, E. J., Shindano, J., & Linnemann, A. (2019). Fermented cereal-based Munkoyo beverage: Processing practices, microbial diversity and aroma compounds. *PLoS One*, 14(10): 1-16.

[68] Rahman, M. S. (2020). Food preservation: an overview. *Handbook of food preservation*, 7-18. (3rd edition). CRC Press.

[69] Rahman, M. S., & Labuza, T. P. (2020). Fundamentals of Water Activity Concept. In *Handbook of Food Preservation*, 473-486. CRC Press.

[70] Rolle, R., & Satin, M. (2002). Basic requirements for the transfer of fermentation technologies to developing countries. *International Journal of Food Microbiology*, 75(3): 181-187.

[71] Salama, S. M., & Mariod, A. A. (2022). Significance of African Fermented Foods in Nutrition and Food Science. In *African Fermented Food Products-New Trends* (pp. 37-44). Cham: Springer International Publishing.

[72] Schaeffer, A. B., & Fulton, M. D. (1933). A simplified method of staining endospores. *Science*, 77(1990), 194-194. https://doi.org/10.1126/science.77.1990.194

[73] Sekoai, P. T., Ghimire, A., Ezeokoli, O. T., Rao, S., Ngan, W. Y., Habimana, O.,... & Hung, C. H. (2021). Valorization of volatile fatty acids from the dark fermentation waste Streams-A promising pathway for a biorefinery concept. *Renewable and Sustainable Energy Reviews*, *143*, 110971.

[74] Shields, p., and Cathcart, L. (2012). Motility test medium protocol. http://www.microbelibrary.org/library/laboratory-test-medium-protocol. Last accessed March 6th, 2012.

[75] Singh, B., Kumar, P., Yadav, A., & Datta, S. (2019). Degradation of fermentation inhibitors from lignocellulosic hydrolysate liquor using immobilized bacterium, Bordetella sp. BTIITR. *Chemical Engineering Journal*, *361*, 1152-1160.

[76] Sulieman, A., Esra, A., & Abdelgadir, W. (2015). Isolation and identification of yeasts from the different stages of hulu-mur fermentation. *Journal of Microbiology Research*, 5, 71-76. https://doi.org/10.5923/j.microbiology.20150502.04

[77] Sunano, Y. (2017). Nutritional Value of the Alcoholic Beverage “Parshot” as a Staple and Total Nutrition Food in Dirashe Special Woreda, Southern Ethiopia. Journal of Food Processing and Beverages. 5(1): 12-30.

[78] Tadesse, B. T., Abera, A. B., Tefera, A. T., Muleta, D., Alemu, Z. T., & Wessel, G. (2019). Molecular characterization of fermenting yeast species from fermented Teff dough during preparation of injera using ITS DNA sequence. *International journal of food science*, 2019: 7.

[79] Shields, p., and Cathcart, L. (2022). Motility test medium protocol. http://www.microbelibrary.org/library/laboratory-test-medium-protocol. Last accessed March 6th, 2025.

[80] Tamang, J. P. (2010). Diversity of Fermented Foods. *Fermented Foods and Beverages of the World*, 41-84.

[81] Tamang, J. P., Shin, D. H., Jung, S. J., & Chae, S. W. (2016). Functional properties of microorganisms in fermented foods. *Frontiers in microbiology*, 7: 1-13.

[82] Teklu, B., Gebremariam, G., Aregai, T., & Saripalli, H. R., (2015). Determination of Alcoholic Content and other Parameters of Local Alcoholic Beverage (Tella) at Different Stages in Gondar, Ethiopia. International Journal of IT, Engineering and Applied Sciences Research, 4(6): 37-40.

[83] Tibbetts, S. M., Mann, J., & Dumas, A. (2017). Apparent digestibility of nutrients, energy, essential amino acids and fatty acids of juvenile Atlantic salmon (Salmo salar L.) diets containing whole-cell or cell-ruptured Chlorella vulgaris meals at five dietary inclusion levels. *Aquaculture*, 481: 25-39.

[84] Umar, M., Mohammed, I. B., Abdulkarim, I. M., Yusuf, G., Yaya, A. A. and Leo, G., (2016). Comparative studies on the prevalences of salmonella species in two homemade fermented beverages (zobo and kunun-zaki) sold at samara, zaria, kaduna, Nigeria. International Journal of Scientific and Research Publications, 6(3): 428-435.

[85] Wassie, T., & Wassie, M. (2016). Isolation, Characterization and Identification of Lactic Acid Bacteria from Ready to Consume Shamita: Ethiopian Traditional Fermented Beverage. *International Journal of Life Sciences and Technology*, 9(6): 51-55.

[86] Walker, G, M., Stewart, G. G. (2016) Saccharomyces cerevisiae in the Production of Fermented Beverages. 2. https://doi.org/10.3390/beverages2040030

[87] Wedajo Lemi, B. (2020). Microbiology of Ethiopian traditionally fermented beverages and condiments. *International journal of microbiology*, 2020: 8.

[88] Xiang, H., Sun-Waterhouse, D., Waterhouse, G. I., Cui, C., & Ruan, Z. (2019). Fermentation-enabled wellness foods: A fresh perspective. *Food Science and Human Wellness*, 8(3): 203-243.

[89] Yohannes, T., Fekadu, M., Khalid, S. (2013). Preparation and physicochemical analysis of some Ethiopian traditional alcoholic beverages. *African Journal of Food Science*, 7(11): 399-403.

[90] Zabed, H., Faruq, G., Sahu, J. N., Azirun, M. S., Hashim, R., & Nasrulhaq Boyce, A. (2014). Bioethanol production from fermentable sugar juice. *The scientific world journal*, 2014.

[91] Zakpaa, H, D., Al-Hassan, A., and Adubofour, J. (2010). An investigation into the feasibility of production and characterization of starch from “apantu” plantain (giant horn) grown in Ghana. *African Journal of Food Science.* 4(9): 571 - 577.