College of Natural Sciences

Department of Biology

Microbiology and proximate composition analysis of wacatha and Ogoli traditionally fermented beverage produced in Abobo district, Gambella, Western Ethiopia

By

Lero Obang (MSc)

A Thesis Submitted to the Department of Biology, College of Natural Sciences, Jimma University, In Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology (Applied Microbiology)

Jimma, Ethiopia

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List of Acronyms

AMB	Aerobic Mesophilic Bacteria
AFB	African Fermentation Beverages
AFCB	Africa Fermented Cereal Beverages
MRS	de Man Rogosa Sharpe
FB	Fermented beverages
FBP	Fermented Beverage Products
FPM	Foodborne Pathogens Microorganisms
LAB	Lactic Acid Bacteria
MSA	Mannitol Salt Agar
PCA	Plated Plate Agar
PDA	Potato Dextrose Agar
TFB	Traditional Fermentation Beverages
TFP	Traditional Fermentation Processes
TFD	Traditional Fermented Drink
WTFB	Wacatha Traditional Fermented Beverage
OTFB	Ogoli Traditional Fermented Beverage

Abstract

The traditional fermented beverage has advanced both low-income consumers as well as women in terms of revenue generation and are largely viewed as being a major health problem due to contamination. The objective of this study was to assess the Microbiology and proximate composition analysis ofwacatha and Ogoli traditionally fermented beverage produced in Abobo district, Gambella, Western Ethiopia. A cross-sectional design was employed for socio-demographic and sample collection. Wacatha and Ogoli vendors were considered as respondents. Standard methods were used for the enumeration of *aerobic mesophilic bacteria, Enterobacteriaceae, coliform, aerobic bacterial spore, staphylococci, lactic acid bacteria, and yeasts.* A total of 60 (30 each of wacatha and ogoli) samples were collected from Abobo district. Data were analyzed using SPSS software version 20.0. The result of the sociodemographic study showed, 80% of wacatha venders were women. 70% of ogoli were male, about 46.7% of wacatha vendors were in secondary, 50% of ogoli were in a primary. About 93.3% of wacatha and 100% of ogoli did not use special cloth for selling. The mean microbial count of (log CFU/ml) of Wacatha sample were dominated by *Yeasts (7.17±0.037), Aerobic mesophilic bacteria (7.05±0.24), Coliform (6.62±0.39), Staphylococcus (5.85±0.34), LAB (5.02±0.35), Enterobacteriaceae (Entero) (4.67±0.53),* and *spore-forming bacteria (ASFB) (1.56±0.13).* But *Ogoli was dominated by yeast (7.08±0.21), AMB (6.94±0.20), coliform (6.67±0.37), Staph (6.60±0.27), LAB (5.59±0.14), Entero (5.02±0.48),* and *ASFB (2.47 ±0.06).* A total of 606 isolates, 314 isolates from Wacatha, and 292 isolates from Ogoli were characterized and grouped into 15 genera with 413 rods and 193 cocci shapes, the microbial dynamic in both Wacatha and Ogoli at the beginning were dominated by Entero, AMB, and Coliform while at the end of fermentation was dominated by yeasts. The physicochemical parameters in Wacatha were pH (3.84±0.24), Temperature (19.7±1.8), Titratable (1.37±0.23), Alcoholic (3.4±0.15), Moisture (94.3±0.4), Total ash (0.25±0. 51), fat (0.25±050), Total protein (0.70±0.1 5) and carbohydrate (4.4±0.41). Whereas Ogoli pH (3.76±0.26), (15. 6±0.34), Titratable (1.31±0.25), Alcoholic (3.3±0.10), Moisture (94.9±0.39), ash (0.20±0.11), fat (0.24±0.05), protein (0.65±0.17), and carbohydrate (4.5±0. 61). The high count of microbial in the two beverages of poor-quality indicators could be due to poor personal hygiene, processing, and materials. Largely, the microbial load of beverages was over the limit so a call for attention is mandatory.

Keyword. Beverage, Fermentation, microbiology, microbial dynamic, Proximate

1. Introduction

1.1 Background of the study

Fermentation is one of the effective and the most economical methods of processing and preserving of beverages (Bacha *et al.*, 1999). Traditional fermented beverages are created by indigenous knowledge passed down from generation to generation which requires a range of locally accessible substrates. As a result, their industrial operations rely on old techniques and inadequate equipment that can be found locally (Kebede *et al.*, 2002). Fermented beverages vary according to society, geography, time, components, and the microorganisms involved (Law, *et al.*, 2011).

Fermented beverages also are a source of food and social importance in both developed and developing countries. As a result, many households in the country, especially women, engage in the manufacturing and sale of fermented beverages as their primary source of revenue to sustain themselves and their families (Tafere, 2015). Fermentation, which is typically regarded as a low-input enterprise, delivers safe, low-cost, and nutritious foods to people with limited purchasing power (FAO, 2012) which is inversely used for medicinal reasons, such as improving food digestibility, improving food flavor, and degrading hazardous organisms, as well as for recreational ones, such as weddings, religious and nonreligious events (Anteneh *et al.*, 2011; Kohajdova and Karovicova, 2007).

In the world, some types of alcoholic beverages native to regions were prepared and consumed similarly in Africa, traditional fermented beverages are consumed in different occasions such as marriage, naming and rain-making ceremonies, at festivals and social gatherings, at burial ceremonies and settling disputes *(*Sahle and Gashe 1991), In Ethiopia, there are available many traditional fermented beverages such as tej, borde, tella, shameta, korefe, keribo, siljo, grawa, and others (Nemo and Bacha 2020). Likewise, wacatha traditional fermented beverages (WTFB) and ogoli traditionally fermented beverages (TFB) are prepared and consumed in the Gambella region, especially during holidays or any special event. But now a day they are becoming fermented regularly as a source of income.

Isolation of microorganisms involved in the fermentation of cereal would be important to support the technical process and to achieve an expectable end product with the desired quality (Nwachukwu *et al*., 2010) for instance according to Mogessie (2006) report, the fermentation of beverages are carried out by microorganism particularly lactic acid bacteria (LAB) and Yeasts, and as also stated by Nemo and Bacha (2020) that yeast (6.31 ± 0.63) and lactic acid bacteria (6.09 ± 0.53 log CFU/ml) in tej mean microbial count was dominant respectively.

Microbial dynamic of traditional fermentation of beverages, several groups of microorganisms are known to involve in the fermentation processes. While some microbes initiate the fermentation process, others dominate the fermentation process in succession until few strains finally takeover the remaining fermentation phases to ward the end of fermentation (Kosisochukwu *et al*. 2020; Mulaw and Tesfaye 2017) due to the accumulation of different metabolites and inhibitory substances produced as by-products provide an appropriate and conducive environment to less sensitive species while it inhibits the sensitive pathogens through the production of inhibitory factors (Chaves-Lopez *et al.* 2020).

Fermentation contribute to the lowering of pH resulting in suppression of growth of some unwanted microbes, improvement in organoleptic properties the fermenting mash, and produce beneficial compounds. Such changes make fermented beverages good sources of energy and ideal products for consumption by majorities of the population (Phiri *et al.,* 2019). Hence, investigating the properties of many of the traditionally fermented beverages is neecessary to understand their nutritional benefits and production processes (Romero-Luna *et al.,* 2017). Nutritional composition of beverages have impacts on the type and rate of microbial spoilage. In general, all ingredients that provide nutrients for microbes increase the product susceptibility to spoilage. Beverages differ greatly in their nutritional status (Stratford 2006).

Many traditional fermented beverages in Ethiopia have been studied in different areas in the region, however, due to preparation, ingredient, and place to place, as well as microbiology and proximate composition in each fermented beverages are varied as a result yet no related studies were done on wacatha and ogoli traditional fermented beverage microbiology and proximate composition analysis, Therefore, the purpose of this study was, therefore; initiated to assess the microbiology and proximate composition analysis of some traditionally prepared beverages in Abobo district, Gambella, western Ethiopia

1.2 Objective the study

1.2.1 General objective

- To assess the Microbiology and proximate composition analysis of some traditionally prepared beverages in Abobo district, Gambella, western Ethiopia.

1.2.2 Specific objectives

- To evaluate the knowledge and practice of Wacatha and Ogoli brewing protocol
- To determine Wacatha and Ogoli associated microbes and identify their common genera
- To determine microbial dynamics of the two beverages
- To investigate the physicochemical properties of the two beverages
- To evaluate the nutritional content of Wacatha and Ogoli beverage

1.3 Statement of the problem

Based on the diversity in fermented beverage types in the country make a variety of the traditional fermented beverages and the microbiology of Ethiopian beverages remains to be studied (Tafere, 2015). Nutritional composition of beverages have impacts on the type and rate of microbial spoilage. In general, all ingredients that provide nutrients for microbes increase the product susceptibility to spoilage(Stratford, 2006). The problem of sanitation and personal hygiene during fermentation facilitates beverage toward spoilage or contamination (Rashid *et al*., 2013). some physical aspects such as temperature, relative humidity, and aeration, are often poorly controlled and production techniques are not standardized (Rolle and Satin, 2002).

1.4 Significance of the study

The importance of this study on the community is to make people familiar with the microbial quality and physicochemical properties, documenting Wacatha and Ogoli as an Ethiopia traditional alcoholic beverage that will be used in a wide range of foods marketing both in developing and further study for industrialization, extension services to introduce some improved methods, particularly those for hygiene and safety and to give hint about microbial quality, the nutritional value of both Wacatha and Ogoli traditional fermented beverages

2. Literature Review

2.1 Fermentation

Fermentation is the oldest method of food preservation that is employed in traditional cultures and village life. Women are thought to have created fermentation techniques over time to preserve food for times of famine, provide desirable flavor to foods, and lessen toxicity (Rolle and Satin, 2002) Fermentation is still widely used as a household or village-level technique in many countries today (Holzapfel, 2002).

Fermentation has enhanced the shelf-life and safety of foods and beverages, allowing our predecessors in temperate and cooler areas to survive the winter season and those in tropical climes to survive dry seasons. Fermented foods are valued as important dietary components in many developing countries. The enormous variety of foods marketed in both emerging and developed countries, not only for the benefit of preservation and safety but also for their highly-valued sensory characteristics, demonstrates the relevance of fermentation in modern life (Rolle and Satin, 2002).

Fermented products can play an important role in contributing to the livelihoods of rural and peri-urban dwellers alike, through enhanced food security, and income generation via a valuable small-scale enterprise option. There is such a diversity of fermentable substrate available year-round, that the activity can provide a regular income. Although harvesting or substrate may be seasonal, fermentation itself is largely independent of weather, and byproducts can be recycled into livestock fodder. Fermentation activities are highly combinable with a variety of other traditional and domestic activities and can make a particularly important contribution to the livelihoods of women, the disabled, and landless poor who, with appropriate training and access to inputs, can increase their independence and self-esteem through income generation (FAO, 2012).

2.2 Diversity of fermentation products

There may be over 5000 different types of fermented foods and alcoholic beverages enjoyed by billions of people throughout the world today as staples and other culinary components (Tamang, 2010b). Alcoholic beverages are all classified into nine major groups based on the substrates

used from plant/animal sources such are fermented cereals, fermented vegetables, and bamboo shoots, fermented legumes, fermented roots/tubers, fermented milk products, preserved meat products, dried and smoked fish products (Tamang, 2010c).

Various scholars have studied the changes in local traditional fermented food and beverage products at various times. Bearing in mind the rich diversity in fermented food and beverage types in the country; few studies were carried out in widely different parts of Ethiopia and included the major ethnic groups (Mulaw and Tesfaye, 2017). The major indigenous fermented beverages and foods which are produced in Ethiopia are Borde, Shamita, Tella, Tej, Araki, Korefe, Keribo, Duka, and others (Wassie, 2016),

2.3 Traditional fermented beverages in Ethiopia

Ethiopia is a country rich in traditional fermented beverages in history, ethnicity, and cultural and crop genetic diversity. Traditional alcohol beverages are widely consumed among different ethnic groups of the country as a prominent part of the local traditions of major social events including public holidays, weddings, funerals, and other forms of festivities (WHO, 2014).

Fermentation of Ethiopian drinks is rather simple and does not necessitate the purchase of expensive equipment. It is customary to use and follow a controlled natural fermentation process during the creation of traditional fermented food products, with no specific starter cultures needed to initiate it. Ethiopian local fermented beverages are a product of an acid-alcohol type of fermentation which the preparation of many local fermented beverages is still practiced in the household that known to enjoy a wide range of fermented foods and beverages. (Mulaw and Tesfaye, 2017). Some of the most widely consumed home-brewed beverages in Ethiopia are Tella, Shamita, Tej, Borde, and Areki. Of these traditionally fermented beverages of Tej, Tella and Areki are the most preferred drinks for big festive occasions (WHO, 2014).

2.3.1 Wacatha traditional fermented beverages

Wacatha is one of the known traditionally fermented beverages in Gambella, western Ethiopia. It is a commonly consumed and prepared beverage in a different area of the region especially during holidays or any other special event. Wacatha is prepared and consumed in a time interval period of twenty-four hours and mostly consumed by adult people as its alcoholic fermented beverage due to this it is consumed by those who usually use it to other alcoholic drinks. The

basic ingredients used for the preparation of *Wacatha* are cereal, malt, honey, water, and enkuro respectively

2.3.2 Ogoli traditional fermented beverages

Ogoli is another traditional fermented beverage prepared and consumed in Gambella particularly in the Anywaa zone and Majang. The ingredient used for fermentation of Ogoli is honey, water, and Agiima (done by scraping the hardcover part of the Aromo plant and putting it onto the pot within the water with frequencies exchange of water followed with wash and finally, after five days more the (Agiima) honey would be added simply for attempting until the Agiima become good for fermentation. And Ogoli could be fermented and consumed in three to four hours but mostly the duration of fermentation depends on the good of the Agiima.

2.4 Ingredients

2.4.1 Physicochemical properties of honey

Honey is a substance that has been used for eras to make brews which used to produce different types of beverages that may contain different flavors depending on the flowery sources of honey in which its quality is known by their sensory, physical, and chemical properties. Its physical and chemical properties depend on the nectar, pollen floral source, color, flavor, moisture, protein content, and sugar (Da Silva *et al.*, 2017).

All kinds of honey share certain general characteristics, including moisture content below 20%, the sugar content of 70-80%, an ash content ranging from 0.1% to 0.2%, and a pH between 3.8 and 4.7 and Honey contains proteins, free amino acids (main proline), organic acids, aromatics, and vitamins and mineral are minor components and several enzymes are important components of honey such an α-glucosidase, β-glucosidase, amylase, and glucose oxidase. The specific percentages of all these different components may vary depending on the plant origin, the geographical location, the season in which the honey is collected, the treatment of honey since its harvesting, and its age (Da Silva *et al.*, 2017).

2.4.3 Malt, Enkuro and Agiima preparation

According to Hotessa, and Robe, (2020) barley is important for the preparation of malt. However, the processing steps are not markedly different. For malt preparation, barley is cleansed to remove dirt and extraneous materials and steeped in water for about a day. Excess

water is drained off, and the soaked barley is allowed to germinate for five days wrapped in banana leaves. Later, germination barley can be sun-dried and ground finely

Enkuro preparation was done by grounded the maize grain either by grinding stone or hammer mills to the floor and after grounded floors mixed with water in a solid-state and keep it in a big pot for about one week until it became sour with frequent checking thereafter the sour floor roast on grill plate then uses or hold sun dry until use. Agiima is the ingredient used as a starter culture for Ogoli fermentation which was made by scraping the hardcover of the Aromo plant and putting it into the pot on the water with frequencies exchanges of water after one or two days followed with wash finally, after five days more the (Agiima) honey would be added simply for attempting until the Agiima become good for fermentation.

2.5 Microbiological Quality

2.5.1 Aerobic mesophilic Bacteria

Aerobic mesophilic Bacteria is significant in food microbiology as an indicator of the microbiological quality as well as a measure of sanitation used during the preparation and handling of food and beverages. Higher load of bacterial spoil the food quicker and Cause loss of quality of food (Jacob, 2010).

2.5.2 Enterobacteriaceae

Enterobacteriaceae is gram-negative, facultatively anaerobic bacteria that include 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, in the digestive tracts of animals and are which are commonly isolated from foods and beverages. Enterobacteriaceae species are the high most probable number prove clearly that poor hygiene meals could be sources of food-borne disease (Motarjemi *et al.*, 1993).

2.5.3 Staphylococcus species

Staphylococcus aureus is gram-positive, *cocci* shape, non-motile, non-spore former, facultatively anaerobic, Catalase mostly positive and contaminate food products during preparation and processing, it remains a major source of foodborne sickness. It is a normal flora of humans and

animals which lives 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 (Schmitt *et al.,* 1990).

2.5.4 Bacillus species

Bacillus species 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 (Aruwa and Olatope, 2015).

2.6 Microflora 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 (Tafere *et al.,* 2015). Spontaneous' fermentations typically result from the competitive activities of different microorganisms; strains best adapted and with the highest growth rate would dominate during particular stages of the process. Traditional beverages are typical examples of lactic fermentation followed by alcoholic fermentation in which initially lactic acid bacteria and later *yeasts* play the dominant role (Holsapfel, 2002).

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* (Djè *et al.,* 2009). The oldest and most economically relevant biotechnology is yeast's generation of alcoholic beverages from fermentable carbon sources 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 (Walker, 2016).

Yeasts play an important role in the creation of African traditional fermented foods and beverages particularly *Saccharomyces cerevisiae* has been called "mankind's most domesticated organism (Mogmenga, 2019). Lactic acid bacteria are widely distributed in nature and able to promote fermentation by utilization of food nutrients and producing a variety of substances

(O'Shea *et al.,* 2011). Lactic acid bacteria are Generally Recognized as Safe and can be used well for medical and veterinary applications (Hoque *et al.,* 2010).

Table 1. Dominants of microorganisms in various fermented beverages

Beverages	Microorganisms involved in fermentation	References
Borde	*Enterobacteriaceae, Leuconostoc mesenteroid, Bacillus, Micrococcus, Streptococcus, Weissella confusa, Lactobacillus Brevis, Lactobacillus viridescens, Pediococcus pentosaceus, Lactobacillus curvatus, Lactobacillus collinoides, Lactobacillus sanfrancisco, Lactobacillus pontis, and yeast (Saccharomyces).*	(Gizaw,2018 and Abegaz, 2002)
Keribo	*leuconostoc mesenteric and Aerobic mesophilic bacteria, and yeasts*	Abafida, (2013)
Tej	*Lactobacillus sp. (mainly Lactobacillus Plantarum), Leuconostoc, Pediococcus sp., and Streptococcus. cerevisiae, Debaromyces phaffi, and yeasts*	Hunduma, (2013)
Korefe	*bacillus spp, Micrococci, Lactobacillus, Leuconostoc, Yeast*	Lemi, (2020)
Shamita	*Pediococcus, Lactococcus, Staphylococcus, Streptococci, Staphylococci, Saccharomyces and Rhodoturula spp., and Molds*	Tesfaye, (2018).

2.7 Importance of socio-cultural and economic fermented beverages

Traditional fermented beverages are created by the spontaneous non-alcoholic or alcoholic fermentation of value from germinating cereals such as Sorghum, maize, and millet which are grown and consumed in almost every corner of Africa. Many traditional fermented foods and beverages are still prepared as domestic art in Africa, where they have an important socio-cultural and economic significance (Chelule *et al.,* 2010).

Traditional fermented beverages are frequently associated with the traditions of hospitality and kindness, and they are a component of most families' etiquette. They help individuals form harmonious connections (N'kwe *et al.,* 2005). Women are now playing an increasingly crucial role in the creation of traditional African beverages. Despite differences in technology from

country to country and region to region, African traditional beverages share many of the same characteristics: a short shelf-life, no or low alcohol, a sour flavor, solids and microorganisms in suspension, as well as taste and color characteristics, all at a low cost and widespread availability in some populations (De Lemps. 2001).

2.8 Nutritional aspect of beverage

In numerous African countries, traditional cereal fermented beverages are consumed as either food or drink because they satisfy hunger, and also because of their high nutritional value. Traditional cereal fermented beverages are important nutrients used as a source of energy which often provides to contributes to the diet of the population (Abdoul-Latif *et al.,* 2013). Thus, they contain a high percentage of starch, sugars, and proteins. They are also sources of B-group vitamins (thiamine, riboflavin, and niacin) and minerals such as iron, manganese, magnesium, phosphorus, calcium, potassium, and copper (Michodjèhoun *et al.,* 2005).

Fermented foods are frequently higher in nutrients than unfermented ones. There are at least three ways to accomplish this. Microorganisms are both catabolic and anabolic, synthesizing multiple complex vitamins and other growth factors in addition to breaking down more complex molecules. The liberation of nutrients held in plant structures and cells by indigestible components is the second significant method in which fermented foods can be nutritionally improved (Hasan *et al.,* 2014).

Fermentation can improve food safety by inhibiting pathogenic bacterial growth, toxin breakdown, and improving the shelf life and digestibility of raw food ingredients, the preservative nature of *LAB* species involves the ability to block the growth of pathogenic microorganisms by nutrient competition and bacterial inhibit or production. Some of the inhibitors include organic acids, hydrogen peroxide, and bacteriocin. Lactic and acetic acids are particularly effective at inhibiting the growth of gram-negative bacteria, where hydrogen peroxide has a strong oxidizing effect on most pathogenic bacteria (Borresen *et al*., 2012).

2.9 Health benefit aspects of beverage

Many African fermented drinks (AFB) have medicinal properties with health benefits that are derived either directly from the interaction of eaten live microorganisms (bacteria or yeast) or indirectly from the consumption of microbial metabolites created during the fermentation process (Oyewole and Isah, 2012).

According to much research, the beneficial bacteria found in fermented foods enhance and aid the digestive system's assimilation of food, resulting in greater nutrition and hence increased immune system efficacy. Fermentation also raises the acidity of beverages that avoids spoiling and can rid food of dangerous germs, additionally, antibiotics and bacteriocins produced by certain lactic acid bacteria have been discovered to prevent spoilage and pathogen bacteria that cause diseases (Marshall and Mejia, 2011).

According to Lei *et al.*, (2006) report, traditionally fermented drinks (TFD) have a significant role in preventing acute diarrhea, and according to Nyanzi and Jooste, (2012) report, Kunun-Zaki beverage was popularly believed to enhance lactation in nursing mothers. According to Tamang and Kailasapathy, (2010) studies showed that traditional fermented beverages contained antioxidant components, such as vitamin C and phenolic compounds that are important in maintaining health and protection from coronary disease and cancer. Several vitamins B including niacin (B3), pantothenic acid (B5), folic acid (B9), and also vitamins B1, B2, B6, and B12 are released by lactic acid bacteria in fermented foods. These vitamins are co-factors in some metabolic reactions (Nyanzi and Jooste 2012).

2.10 Traditional fermented beverage safety

Traditional fermentation processes are often uncontrolled and reliant on microorganisms from the environment or the fermentation substrate to start, resulting in poor yield and variable quality products. However, Fermented foods are recognized as major nutritional components in many developing countries because, when done properly, fermentation enhances food safety in an old process while also extending food storage quality under ambient settings. (Holzapfel, 2002).

Fermentation is typically thought to increase the sanitary quality and safety of foods, but if it fails, spoiling can occur and pathogens might persist, posing unexpected health hazards in mostly

safe food products. The utilization of starter cultures chosen based on multifunctional factors can considerably improve the quality, safety, and acceptance of traditional fermented foods. (Holzapfel, 2002).

2.11 Contamination associated with beverage and consumption

Food contamination is defined as the presence of harmful chemicals and microorganisms in food and beverages which can cause consumer illness. These chemicals and organisms may be introduced into food at various stages: production, processing, marketing. Because of its adverse health effects, food contamination has become a matter of serious concern all over the world especially in developing countries (Rafiq and Shah, 2015).

Homes made traditional fermented beverages are usually under unhygienic conditions to the micro flora as the main sources of contamination are humans, sewage, raw materials, utensils, processing equipment, and environment, poor handling and storage conditions, and rodents (Eze *et al.*, 2011).

3. Materials and methods

3.1. Description of the study site and period

Gambella geographical coordinates are between 3º10'– 35º 50'N and 8º15'-34º35'E with an altitude 526 m above sea level. Gambella mostly is flat and its climate is hot and humid and annual rainfall averages about 600 mm with minimum-maximum temperatures are approximately 21.1^{0}C and 35.9^{0}C respectively. According to central statistical agency (2007) Gambella region has a population of 306,916 people and is predominantly inhabited by five indigenous ethnic groups, namely the Anywaa, Nuer, Majang, Opo, and Kumo, and other highlanders. Their main agriculture mostly crops particularly maize, sorghum, and livestock such as cattle and sheep (Abraham, 2002). Gambella has many different types of traditional fermented beverages with different preparation but the most commonly consumed and prepared cereal beverages are Wacatha, Ogoli, Borde, Olango, Acotha, Araki, and Abiethkimera these fermented beverages are important as a food replacement and social gathering or any special event.

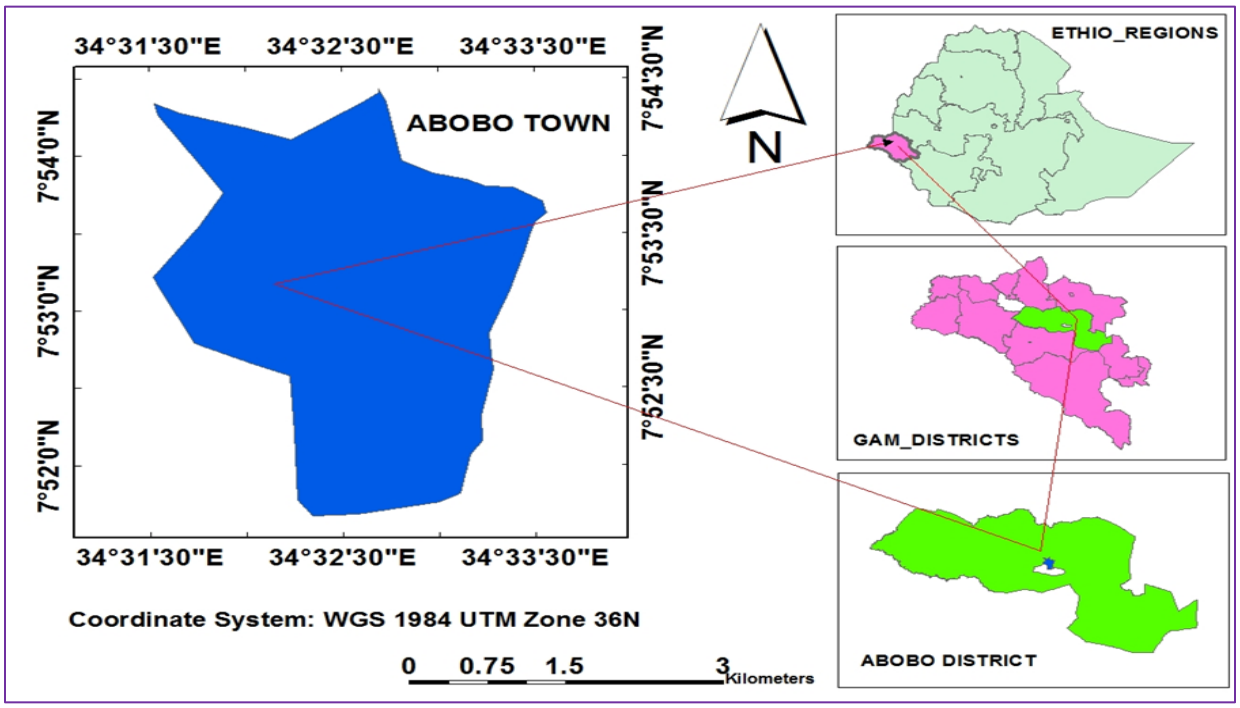

Figure 1. Map showing the study district.

3.2. Study design and Population

A cross-sectional design was employed for socio-demographic and sample collection, microbial and proximate composition analysis. Wacatha and Ogoli makers (vendors) are considered as respondents.

3.3. Socio-demographic characteristics

The socio-demographic characteristics of Wacatha and Ogoli vendors related practice, general sanitation, and handling of the vendors were collected using structured interviews, questionnaires, and observation (appendices.1,2, and 3).

3.4.Sample size and technique

The total populations of the three sites that were engaged in Wacatha and Ogoli vending are 72 irrespective of their age and sex, with the proportion of 30 from Abaaru, 24 from Wangkaak, and 18 from Kano. So, the sample size was calculated by using Cochran (1977) formula.

$n = \frac{n_0}{1+\frac{n_0}{N}}$ Where $n_0 = \frac{Z^2 a/2P(1-p)}{d^2}$ n= total sample size

D= margin of error

N= Total number of the population

p= proportion of population

α= level of significance d = 0.05, P = 0.5 and a = 0.05 $n_0 = \frac{(1.9)^2 \times 0.5 \times 0.5}{(0.5)^2} = 384$

Considering the population correction factor into account the sample size was:

$$n = \frac{384}{1+\frac{384}{72}} \approx 60$$

From the total population, sample size of Abaaru ≈ 24 and Wangkaak ≈ 20, and Kano ≈16. All though population size are small, using formula is necessary to give the correction samples size

3.5. Samples collections

A total of 60 samples of Wacatha and Ogoli samples (30 for each Wacatha and Ogoli) were collected from September–November 2021 randomly from vendors of the Abobo district. About 500 ml of each sample was added into sterile glass bottles with proper label and transported using icebox to Postgraduate and Research Laboratory, Department of Biology, Jimma University, and the samples were kept at 4^{o}C for week for analysis.

3.6. Sample preparations

A 25 ml sample was added in flasks containing 225 ml saline solution (0.85 NaCl /100 ml distilled water) and kept on a rotary shaker at 125 rpm for 5 min. After preparing 10^{-1}-10^{-7} and volume of 0.1 ml aliquot of appropriate dilution of 10^{-3}-10^{-5} was spread-plated on pre-solidified plates and incubated at appropriate temperature and time for enumeration of different microbial groups including aerobic mesophilic bacteria, Enterobacteriaceae, coliform, aerobic bacterial spore, lactic acid bacteria, staphylococci, and yeasts

3.6.1 Aerobic mesophilic bacteria count

AMB was determined after 0.1 ml aliquots of serially diluted sample spreaded on pre-solidified PCA medium and incubated at 32°C for 48 hours (Weil *et al.,* 2006).

3.6.2 Enterobacteriaceae count

Enterobacteriaceae was determined after 0.1 ml of aliquots of serially diluted sample spreaded on pre-solidified Violet Red Bile Glucose medium and incubated at 32^{o}c for 18-24hrs (Spencer *et al.,* 2007).

3.6.3 Coliform Count

Coliform Count was determined after 0.1 ml of aliquots of serially diluted sample spreaded on pre-solidified Violet Red Bile medium and incubated at 32^{o}c for 18-24hrs (Weil *et al.,* 2006).

3.6.4 Aerobic spore-forming bacteria counts

Aerobic bacterial spore counts, 10 ml of the serial dilutions were heated in a water bath kept 80°C for 10 min and then cooled rapidly in tap water. From appropriate dilution, 0.1 ml aliquot

was spread-plated on the pre-dried surface of plate count agar and incubated at 32°C for 72 h (Acco *et al.,* 2003).

3.6.5 Staphylococcus count

Staphylococcus was determined after 0.1 ml of aliquots of serially diluted sample spread on pre-solidified Mannitol salt agar medium and incubated at 37^{o}c for 48hrs (Acco *et al.,* 2003).

3.6.6 Yeast count

Yeast was determined after 0.1 ml of aliquots of serially diluted sample spreaded on pre-solidified potato dextrose agar medium plus one gram of chloramphenicol was dissolved in 10 ml distilled water and incubated at 32^{o}c for 18-24hrs (Spencer *et al.,* 2007).

3.6.7 Lactic acid bacteria count

Lactic acid bacteria were determined after 0.1 ml of aliquots of serially diluted sample spreaded on pre-solidified de Mann Rogosa Sharpe (MRS) agar medium and incubated at 37^{o}c for 48 hours (Patra, 2011).

3.7 Microbial Analysis

After the enumeration of aerobic mesophilic bacteria, Enterobacteriaceae, Coliform, Aerobic bacteria spore count, Staphylococcus, Lactic Acid bacteria, and yeasts, 10 to 15 colonies with distinct cultural characteristics such as colony elevation, margin, color, size, shape, and texture were randomly selected from countable plates and aseptically transferred into a tube containing 5 ml nutrient broth for bacteria or potato dextrose broth for yeasts (Oxoid). Therefore, Aerobic mesophilic bacteria inoculated cultures were incubated at 32^{o}C for 24 hrs., Enterobacteriaceae inoculated culture was incubated at 32^{o}c for 18-24hrs, while Coliform inoculated cultures were incubated at 32^{o}c for 18-24hrs, Aerobic bacteria spore count inoculated culture was incubated 32^{o} C for 72 hours, Staphylococcus inoculated culture were incubated at 37^{o}c for 48hrs, Yeasts inoculated culture was incubated at 32^{o}c for 18-24hrs, and Lactic Acid Bacteria inoculated culture were incubated at 37^{o}c for 48 hrs. The pure isolates were stored on slants at 4^{o}C until further morphological, biochemical, and physicochemical analysis after being purified by sub-culturing. Finally, using John's (2012) bacterial classification manual, the characterization of isolates was done.

3.7.1 Cell Morphological Characterization

The morphological of cell shape, cell arrangement was identified by simple observation, gram staining techniques, motility test, and endospore test to assess the existence or absence of spore

3.7.1.1 Gram staining

Smears of pure isolates were prepared on a clean glass slide and allowed to air dry and heat fix then flooded with crystal violet for one minute and then washed gently with tap water for three seconds. Secondly, smears are rinsed with iodine for one minute, and then washed with tap water. In the third stage, smear slide de-colorization (95%) alcohol and washed in 20 seconds with tap water. Finally, the smear was counterstained by safranin stain and washed after one minute, and stayed put until dry. Thereafter stained slides were examined under oil immersion (100X) to identify isolate-based cell shape or gram-negative bacteria stained pink/red and gram-positive bacteria were stained blue/purple (Gram. 1884).

3.7.1.2 Motility test

A motility medium was prepared using a test tube. A purified broth culture was taken by a sterile needle and stabbed straight vertically into a test tube containing motility medium to the bottom of the tube and incubated at 35°C for 24 hours. A positive motility test was indicated by turbid area diffusing away from the line of inoculation and a negative test was indicated by growth along the inoculation line only but no further (Shields and Cathcart, 2012).

3.7.1.3 Endospore test

According to Schaeffer and Fulton's (1933) method, a smear of isolates was prepared on a clean glass slide and allowed to air-dry and heat-fixed smear was flooded with 0.5 % (w/v) malachite green solution and steamed using cotton dipped in 96 % ethanol for 5 minutes. After cooling, the slide was washed with tap water and counterstained with safranin for the 30s again washed with tap water and allowed to air-dry. Finally; the dried slide was observed under the oil immersion lens (×1000) to assess the presence or absence of endospore.

3.7.2 Biochemical test

3.7.2.1 Potassium Hydroxide (KOH) test

Two drops of 3 % KOH solution were placed on a colony on a clean microscopic slide that was aseptically picked from the surface of nutrient agar using an inculcating loop and stirred in the KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass when the KOH solution become vicious, the thread of slime followed the loop for 0.5 to 2 cm or more in gram-negative bacteria. This test was to identify bacteria based on the presence or absence of slime suspension that follows the loop, the reaction was considered as a negative, and the isolate was considered as gram-positive bacteria (Gregerson, 1978).

3.7.2.2 Citrate utilization

The purified broth was streaked on a slant medium into a test tube and incubated at 37^{o} C for 24hrs to determine citrate utilization as a sole source of carbon to assess growth and color change from green to blue as it was considered as presumptive for Salmonella spp.

3.7.2.3 Catalase test

Catalase test was carried out after smeared overnight grown culture on a clean dry glass slide with 3% H_2O_2 and presence of bubbles considered as Catalase positive (McFadden, 2000), the Catalase test is primarily used to differentiate among Gram-positive cocci. Members of the genus Staphylococcus are Catalase-positive, and members of the genera Streptococcus and Enterococcus are catalase-negative.

3.7.2.4 Urease test

The pure isolate was streaked on a slant and the slant was incubated at 37^{o} C for 24 hours to assess the hydrolysis of urea. Negative was considered as when no color change. (Barnett *et al*., 2000).

3.7.2.5 Triple Sugar fermentation test

The isolated colony was picked from the solid media and streaked the TSIA up to the butt and then streaked the surface of the agar slant then incubated at a temperature of 37°C for 24hours. After the incubation, reddish or yellow coloration indicates acidic or basic utilization. The black

of the butt indicates hydrogen sulfide is produced (H2S), whereas gap spacing of the medium signifies gas formation (Agbagwa *et al*., 2017).

3.7.2.6 Coagulase test

Coagulase test was done using slide test. A colony of the purified isolates was stirred together in a drop of distilled water by two ends of a clean glass slide to make thick suspensions. One was labeled as test and the other was as control. A loopful of human blood plasma was added to one of the suspensions and mixed gently. Clumping within 10 seconds was observed for coagulase-positive organisms (Cheesbrough, 2006).

3.7.2.7 Carbohydrate fermentation test

Was determined to assess the capability of microbes to ferment a specific carbohydrate, 1ml of overnight of LAB and Yeasts were added into a test tube containing 10ml of Phenol Red Broth Base having 2% carbohydrate (Glucose, Sucrose, Maltose) with inverted Durham tube. A fermentation was observed after 3days (72hrs) Gas production, a yellow color taken as positive and Red with no color change taken as negative.

3.7.3 Physiological determination

The Lactic Acid bacteria and Staphylococcus strains isolated from *Wacatha and Ogoli* samples were characterized physiologically based on their capability to grow on 4%, and 6% NaCl, and pH.2 and pH.3 (Kanghae *et al*., 2016).

3.8. Physicochemical parameters of Wacatha and Ogoli samples

Physicochemical parameters of fermented Wacatha and Ogoli such as alcoholic content (ethanol level), pH, Temperature, Titratable Acidity, and Moisture Content of the collected and prepared samples of Wacatha and Ogoli was determined by following standard procedure (Teklu *et al.*, 2015).

3.8.1 pH

The pH of each *Wacatha* and *Ogoli* sample was measured by dipping the electrode of a digital pH meter (microprocessor) into the samples in a measuring cylinder after proper calibration of

the pH meter with a standard solution of pH of 4,7 and 10 buffer accordingly (Yohannes *et al.*, 2013).

3.8.2 Temperature

The temperature of *Wacatha* and *Ogoli* beverages was determined at the site of sample collection by dipping the bulb of mercury glass thermometer into the beverages and the readings were recorded (Umar *et al*., 2016).

3.8.3 Titratable acidity

Titratable acidity was measured by a simple titration method using 0.1ml sodium hydroxide solutions. Ten ml of each sample was measured with a 50 ml measuring cylinder into a conical flask and diluted with 30 ml of distilled water. Three drops of phenolphthalein concentration were added and titrated against 0.1 N NaOH. Then end point indicates by a change from a colorless to a pink solution. The total acidity was calculated as a percentage of lactic acid (Zakpaa *et al*., 2010).

$$\text{Lactic Acid (g/100ml)} = \frac{\text{Amount of NaOH titrated} \times \text{mol/L of NaOH}}{\text{Volume of sample(ml)}}$$

3.8.4 Moisture contents

Moisture content was determined by using 5ml of each sample drying to constant weight in a ventilated thermostatic oven at 110 °C for 3-5hrs and each sample was stay put to cool in desiccators and weighed, the results was calculated by using the following formula as sited (Tigu *et al.*, 1995).

$$\text{Moisture (\%)} = \frac{(\text{Weight of wet mass} - \text{weight of dried mass}) \times 100}{\text{the volume of sample in ml}}$$

Where, $M2$ = Mass of the dish with the dry sample, $M1$ = Mass of the dish with a wet sample, and V = Volume of the sample taken in a test.

3.8.5 Alcoholic content (Ethanol Level)

The alcoholic content of Wacatha and Ogoli samples were measured by direct inserting of alcoholmeter into a 250 ml sample in the measuring cylinder at room temperature after collecting the samples from the site, and the reading was recorded done (ELS, India).

3.9. Proximate Analysis

Determination of nutritional value was achieved by as follows: - Ash content by direct ashing method, crude protein content by automatic distillation and titration system, Crude fat content by simple extraction method, carbohydrate content by calculation (Sunano, 2017), by the following formula: - %Total Carbohydrate = [100 - % (MC%+ Crude Protein + Fat + Ash+ Alcohol)]

3.9.1 Ash content

Ash was determined by igniting a 10ml sample, poured in a crucible, in a muffle furnace 550°C for five hours according to A.O.A.C923.1 (A.O.A.C., 1990). The weight of the sample evaporated and loosed after ignition considered being the weight of total organic matter in the sample, the remaining being the weight of the ash.

Total ash value = $\frac{M2-M1}{V} \times 100.$

Where, M2= Weight of crucible + dry residue, M1= Weight of the crucible, M = volume ml of the sample taken.

3.9.2 Fat content

Fat content was done by simple extraction method through several steps as follow, 10ml of each Wacatha and Ogoli sample and ten ml of distilled water was added into a separatory funnel, Secondly, 25ml peroxide-free ethyl ether was added and corked the tube/funnel and shacked vigorously for 1 minute, Then, next 25 ml of Petroleum ether added and shacked again for 30 seconds and the mixture was stopped for 30 minutes or until separation was completed of Fat and ether layer. Then after completion of the separation of fat and ether layer, the ether layer containing fat was drawn off in an earlier dried and weighed flask. And The extraction was repeated twice and pooled the ether extract, recovers excess solvent and dried the fat for an hour

at 100 ^{0}C, cool and weighed and Fat was dried by keeping the flasks for 30 minutes and weighed, till constant mass is achieved then, cooled for 30 minutes inside a desiccator, and weighed to accuracy in a fine weighing balance.

Crude fat % of diethyl ether extract was calculated by Crude fat = $\frac{M_{1\_}M}{M_2} \times 100$

Where, M1 = Weight in g of Becker and fat residue, M2 = Weight in ml of sample taken
M = in gram of empty Becker

3.9.3 Crude Protein content

Crude protein was done by Automatic distillation and Titration system by following the Procedure sequences, firsts 5ml of each sample accurately measured, to the Kjeldhal Flask, secondly, 3.5 g of K_2SO_4 added on the samples in Kjeldhal Flask, thirdly, 0.1g $CUSO_4$ and 6ml of H_2SO_4 98% Concentrations were added and all samples and chemicals separately mixed in Kjeldhal Flask, mixed samples and chemicals subjected to VELP SCIENTIFICA DK6 Heating digester at 420°C for 60 minutes and finally After digestion the pure digested solution subjected to (VELP SCIENTIFICA MADE IN ITALY UDK159) Automatic distillation and titration system to determine % protein content.

Where by, M1 = Weight in g of Becker and fat residue, M = in a gram of empty Becker and

M2 = Weight in ml of the sample taken (fssai, 2015) (IS: 6287-1985 (Reaffirmed, 2010).

3.9.4 Carbohydrate content

Total carbohydrate content was determined by subtracting the known amounts (g) value of moisture, protein, fat, and total ash (Sunano, 2017).

%Total Carbohydrate = [100 - % (MC%+ Crude Protein +N%+ Fat + Ash %)].

3.10 Fermentation dynamic

Wacatha and Ogoli Microbial successions were assessed by microbiological enumeration during the 24 hours fermentation period for Wacatha, four hours for Ogoli, and Samples were drawn at every 6 hours gap for Wacatha and one hour gap for Ogoli. Accordingly, 10 ml of each sample was suspended in 90 ml of saline solution (0.85g $Nacl_2$ /100ml distilled water) and homogenized in a flask for 10 min using a homogenizer (Edmund Buhler GmbH, Germany) at 100 rpm. Then, 1 ml of each sample was homogenized in 9 ml saline solution and serially diluted up to10^{-1}- 10^{-7}.

Finally, 0.1 ml of aliquot was spread plated on plate count agar (PCA) for aerobic mesophilic bacteria and aerobic spore-forming bacteria (after heating the sample for 10 min at 80 °C for a count of the later), mannitol salt agar (MSA) for staphylococci, violet red bile glucose agar (VRBGA) for Enterobacteriaceae, violet red bile agar (VRBA) for coliform, de Man Rogosa Sharpe (MRS) for lactic acid bacteria, and potato dextrose agar (PDA) supplemented with 200 mgL^{-1} chloramphenicol for yeast and mold.

All of the media were oxoid and incubated at 32 °C for 48 h for bacteria and 28 °C for 2-5 days for yeast and mold. Furthermore, the incubation condition for all microbes was aerobic except for lactic acid bacteria, which was incubated anaerobically using an anaerobic Jar (Hitech e-601, China).

The fermentation of Wacatha and Ogoli beverage under aseptic laboratory from original ingredients were to investigate the microbial dynamic as well the microorganism involved in the ingredients

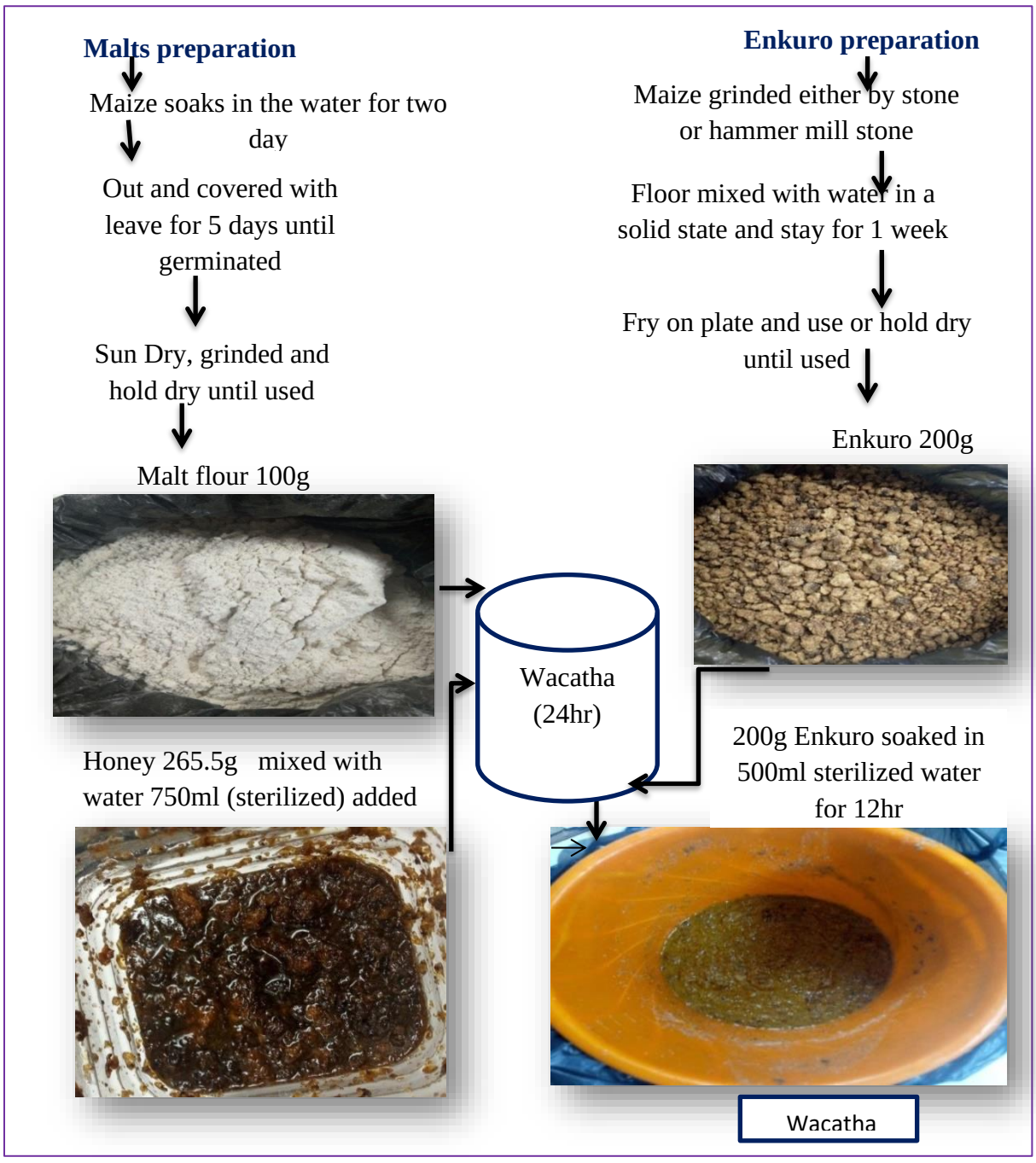

Figure 2. Fermentation of Wacatha beverages under aseptic laboratory procedure

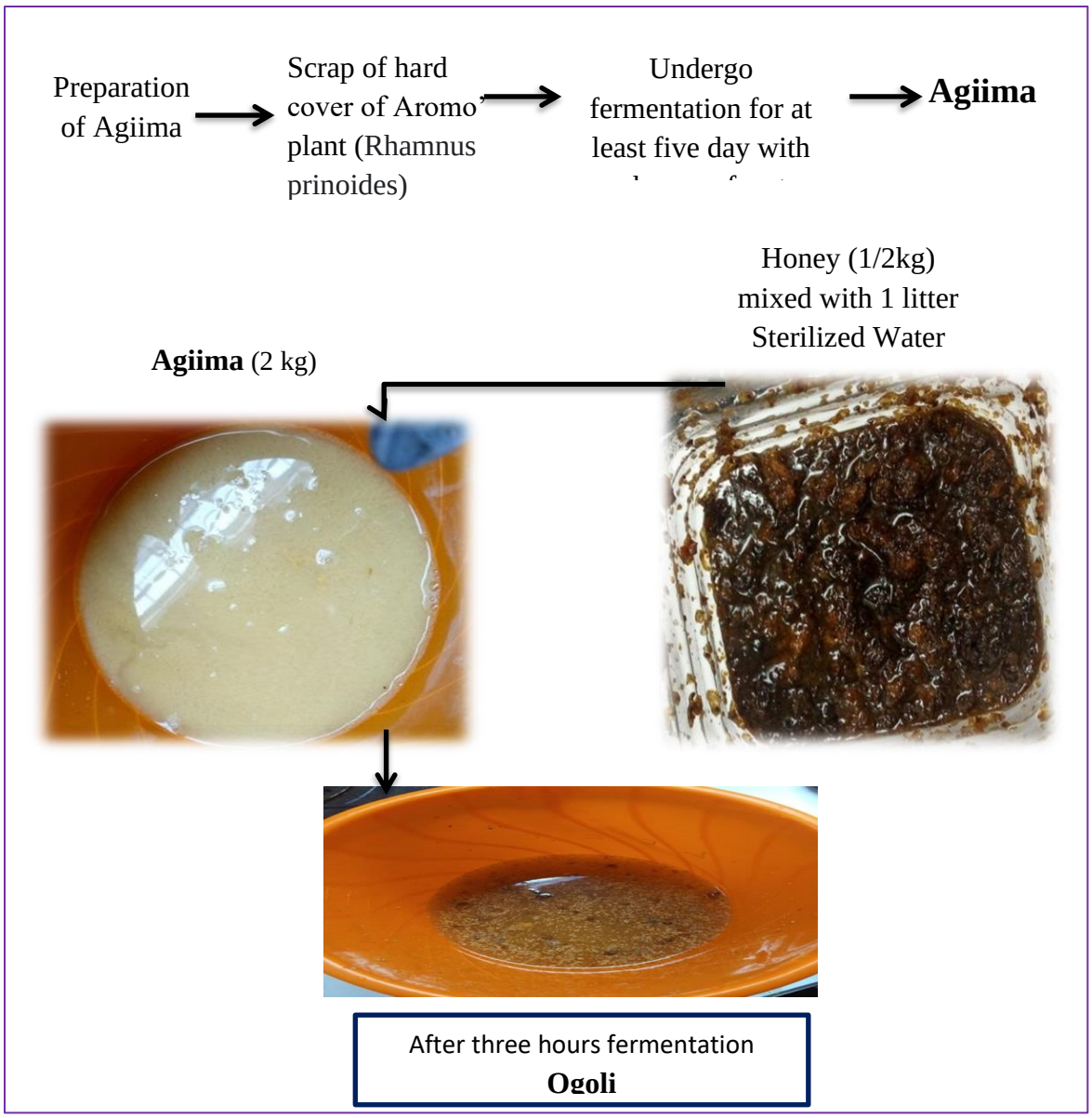

Figure 3. Fermentation of Ogoli beverage under aseptic laboratory procedure

3.10 Data of analysis

Descriptive statistics and one-way ANOVA was used to describe the microbiology count, physicochemical characters, and nutritional value of 'Wacatha' and Ogoli beverages using Statistical Package for Social Science (SPSS) software (version 20.0). microbial growth dynamic curves were analyzed using excel version 2020.

4. Results

4.1. Socio-demographic characteristics of Wacatha and Ogoli vendors

In present study, the majority of wacatha vendors, (36.6%) range between 31-36 ages. Whereas the majority of ogoli vendors (60%) range between 37-43 ages. wacatha vendors about, (20%) were males, and (80%) were females, while ogoli venders about (70%) were males and (30%) were females. wacatha vendors' education levels, (46.7%) were at secondary, and ogoli (50%) were at primary. Experience of wacatha vendors about (43.3%) were 11-15 year and ogoli about (43.4%) were 15-20. (Table.2 and appendix.1)

Tale 2. Socio-demographic characteristics of Wacatha and Ogoli vendors

Parameters	Character	Frequency Wacatha (N=30)	Percentage (%)	Frequency Ogoli (%)	Percentage (%)
Age	25-30	3	10	3	10
	31-36	11	36.6	7	23.4
	37-43	12	40	18	60
	44- 50	4	13.3	2	6.6
sex	Male	6	20.0	21	70.0
	Female	24	80.0	9	30.0
Academic Status	Illiterate	5	16.7	6	20.0
	Primary	10	33.3	15	50.0
	Secondary	14	46.7	8	26.7
	Degree	1	3.3	1	3.3
Experience in vending beverage (years)	< 5	4	13.3	3	9.9
	6-10	6	20.0	4	13.1
	11-15	13	43.3	10	33.3
	16-20	7	23.4	13	43.4

4.2 Knowledge of Wacatha and Ogoli brewers

In present study from a total of 60 respondents (30 for each wacatha and ogoli) showed that 100% of wacatha and ogoli vendors were familiar with fermentation as well as experience. Based on wacatha vendor's experience sources, (53.3%) gained from family while ogoli vendors, about (76.7%) gained experience from relatives (Table.3 and Appendix.2).

Table 3. Knowledge of Wacatha and Ogoli brewers

Characters	**Respondents**	**Frequency of Wacatha**	**Wacatha (%)**	**Frequency of Ogoli**	**Ogoli (%)**
Familiarity with beverage	Yes	30.0	100.0	30	100.0
	No	0.00	0.00	0.0	0.00
knowledge experience	Yes	30	100.0	30	100.0
	No	0.0	0.00	0.0	0.00
Practice knowledge sources	Family	16	53.3	7	23.3
	Relative	14	46.7	23	76.7

4.3 Hygiene practice of Wacatha and Ogoli brewers

In the present study a total of 60 respondents (30 for each wacatha and Ogoli), about (53.3%) of wacatha, and (86.7%) of ogoli vendors stored using shelves. All 100% of both wacatha and ogoli vendors cleaned materials. Based on reagents about, (93.7%) of wacatha and 70% were used reagents. wacatha vendors, about (36.7%) were using Omo and soap while ogoli, about (36.7%) used Omo. wacatha vending place, about (66.7%) of wacatha, (80%) of ogoli had vend for selling. (93.3%) of wacatha and 100% had no coat for selling (Table.4 and appendix.3).

Table 4. Hygiene practice during Wacatha and Ogoli making

Characters	Respondents	Frequency Of Wacatha	Wacatha (%)	Frequency of Ogoli	Ogoli (%)
storage	Bucket	14	46.7	4	13.3
	Shelves	16	53.3	26	86.7
cleaning materials	Yes	30	100.0	30	100.0
	No	0.0	0.00	0.0	0.00
Reagents using	No	2	6.7	9	30.0
	Yes	28	93.3	21	70.0
Types of reagents	Only water	2	6.7	9	30.0
	Omo	11	36.7	11	36.7
	Soap	11	36.7	7	23.3
	Other	6	20.0	3	10.0
Vending	No	10	33.3	6	20.0
	Yes	20	66.7	24	80.0
wear coat	No	28	93.3	30	100.0
	Yes	2	6.7	0.0	0.00

4.4 Mean microbial count (log $CFUg^{-1}$) of Wacatha and Ogoli samples

In the present study, the microbial count (log $CFUg^{-1}$) of AMB in Wacatha samples (7.05 log $CFUg^{-1}$) were highest than in Ogoli (6.9 log $CFUg^{-1}$), while *Enterobacteriaceae in Wacatha (*4.7 log $CFUg^{-1}$) was less than in Ogoli (5.6 log $CFUg^{-1}$), Coliform in Wacatha (6.6 log $CFUg^{-1}$) was highest than Coliform in Ogoli (6.3 log $CFUg^{-1}$), Aerobic spore former Bacteria (ASFB) in Wacatha (1.6 log $CFUg^{-1}$) was less than ASFB in Ogoli (2.4 log $CFUg^{-1}$) In the present study showed that staphylococcus count in Wacatha (5.9 log $CFUg^{-1}$) was less than in Ogoli (6.6 log $CFUg^{-1}$), LAB count in wacatha (5.0 log $CFUg^{-1}$) was the same as in Ogoli (5.0 log $CFUg^{-1}$), and Yeasts in Wacatha (7.2 log $CFUg^{-1}$) was highest than in Ogoli beverages (7.0 log $CFUg^{-1}$). There was a statistically significant difference ($p < 0.05$) among the mean microbial counts in all Wacatha and Ogoli samples between the groups (Table.5 & Appendix.4).

Table 5. Mean microbial count (log CFUg-1) Wacatha and Ogoli samples in Abobo district, Gambella, western Ethiopia.

Types of beverages	No	AMB	% CV	Enter	% CV	Coliform	% CV	ASFB	% CV	Staph	% CV	LAB	% CV	Yeast	% CV
Wacatha	30	7.05±0.2	3.4	4.7±0.5	10	6.6±0.2	6.6	1.6±0.1	5.0	5.9±0.3	5.9	5.0±0.4	5.9	7.2±0.3	5.1
Ogoli	30	6.9±0.2	2.9	5.6±0.1	2.5	6.3±0.4	5.9	2.4±0.06	2.5	6.6±0.3	4.0	5.0±0.5	9.0	7.0±0.2	3.0
Total	60	6.98±0.2	3.1	5.15±0.4	6.2	6.65±0.4	6.2	2.± 0.8	3.7	6.3±0.3	4.9	5.0±0.45	7.4	7.1±03	4.0

Where, *AMB =Aerobic Mesophilic Bacteria, Col=Coliforms; = Staph= Staphylococci; LAB= Lactic acid bacteria; Enter= Enterobacteriaceae.*

4.5 Frequency distribution of dominant Microbes in Wacatha and Ogoli beverages

A total of 606 isolate, 314 isolates from Wacatha and 292 isolates from Ogoli were characterized and grouped into 15 genera and with 413 rods and 193 cocci shape using the standard reference of John's bacterial identification. The predominant microbial group in 30 samples of Wacatha beverages were *Saccharomyces* 44(6.6%), *Lactobacillus* 48(7.2%), *Lactococcus* 43 (6.5%), *Staphylococcus* 25 (3.8%), *Bacillus* 24 (3.6), *Pseudomonas* 20 (3%), *Pediococcus* 37 (5.6%), *Escherichia* 16 (2.4%), *Citrobacter* 15 (2.3%), *Enterobacter* 12 (2.2%), *Streptococcus* 10 (1.5%), *Micrococcus* 8 (1.3%), *Enterococcus* 7 (1.0%), *Providencia* 4 (0.6%), *Proteus* 6(0.9%), *and microbes in Ogoli were Saccharomyces* 60(9%), *Lactobacillus* 45(6.8%), *Staphylococcus* 25 (3.8%), *Bacillus* 24 (3.6%), *Pediococcus* 14 (2.1%), *Escherichia* 22 (3.3%), *Citrobacter* 20 (3%), *Enterobacter* 18 (2.7%), *Streptococcus* 17 (2.6%), *Enterococcus* 16 (2.4%), *Micrococcus* 10 (1.5%), *Klebsiella* 7 (61%), *Providencia 3 (0.5%), Proteus* 5 (0.8%) in Ogoli sample (Table.6 and Appendix.5).

Table 6. Frequency distributions of dominant bacteria genera in samples of *Wacatha and Ogoli* collected, from Abobo district, Gambella region, 2011/2014.

Types of isolates	**Types of the beverages**		**Numbers of isolates (%)**
	Wacatha	Ogoli	
Lactobacillus	48(7.2%)	45(6.8%),	93(14%)
Saccharomyces	44(6.6%)	60(9%)	104(15.6%)
Lactococcus	43(6.5%)	-	43(6.5%)
Staphylococcus	25(3.8%)	25(3.8%),	50(7.6%)
Pediococcus	37(5.6%)	14(2.1%),	27(4%)
Bacillus	2424(3.6)	24(3.6%),	48(7.2%)
Escherichia	16 (2.4%)	22(3.3%),	38(5.7%)
Citrobacter	15(2.3%)	20(3%),	35(5.3%)
Enterobacter	12(2.2%)	18(2.7%)	30(4.9%)
Streptococcus	10(1.5%)	17(2.6%),	27(4.1%)
Micrococcus	8(1.3%)	10(1.5%),	18(2.8%)
Enterococcus	7(1.0%)	16(2.4%),	23(3.4%)
Proteus	6(0.9%)	5(0.8%)	11(1.7%)
Providencia	4(0.6%)	3(0.5%),	7(1.1%)
Klebsiella	-	7(1%)	7(1%)
15	314	292	606

4.6 Microbial dynamics of Wacatha and Ogoli fermentation to consumable

The microbial growth dynamic of Wacatha fermented beverage under laboratory sample was drawn five-time within twenty-four hours at 0hrs, 6hr, 12hr, 18hr, and 24hr, At zero hour fermentation shows that the *Enterobacteriaceae* (5.5log), *aerobic mesophilic bacteria* (5.4log), *Coliform* (5.2log), *Yeasts* (4.9log), *Staphylococcus (*4.6log), *Bacillus* (3.5log), *and LAB* (3.5log), At six hours *Yeasts* (5.4log) dominated the fermentation followed by AMB (4.8log), *Enterobacteriaceae (4.7log)*, *Staphylococcus (*4.7log), *Coliform* (4.8)log, *Bacillus* (4.2log), *and LAB* (3.7log). At twelve hours the microbial growth indicated that *Yeasts* (5.5log) *AMB* (4.8log), *Coliforms* (4.8log), *Staphylococcus* (4.7log), *Enterobacteriaceae* (4.7log), *Bacillus* (3.8log), and *LAB* (3.7log). While at eighteen hours *yeasts* (5.5log) were the leading one followed by *Enterobacteriaceae* (4.8log), *AMB* (4.6log), *Staphylococcus* (4.5log), *Coliforms* (4.5log), *LAB* (3.9log), and *Bacillus (3log).* Finally at twenty-four hours Yeasts (5.5log), *AMB* (4.7log), *Coliform (*4.7log), *Enterobacteriaceae* (4.6log), *Staphylococcus (*4.4log), *LAB* (4.3log), *and bacillus* (3log*) (Figure.7).*

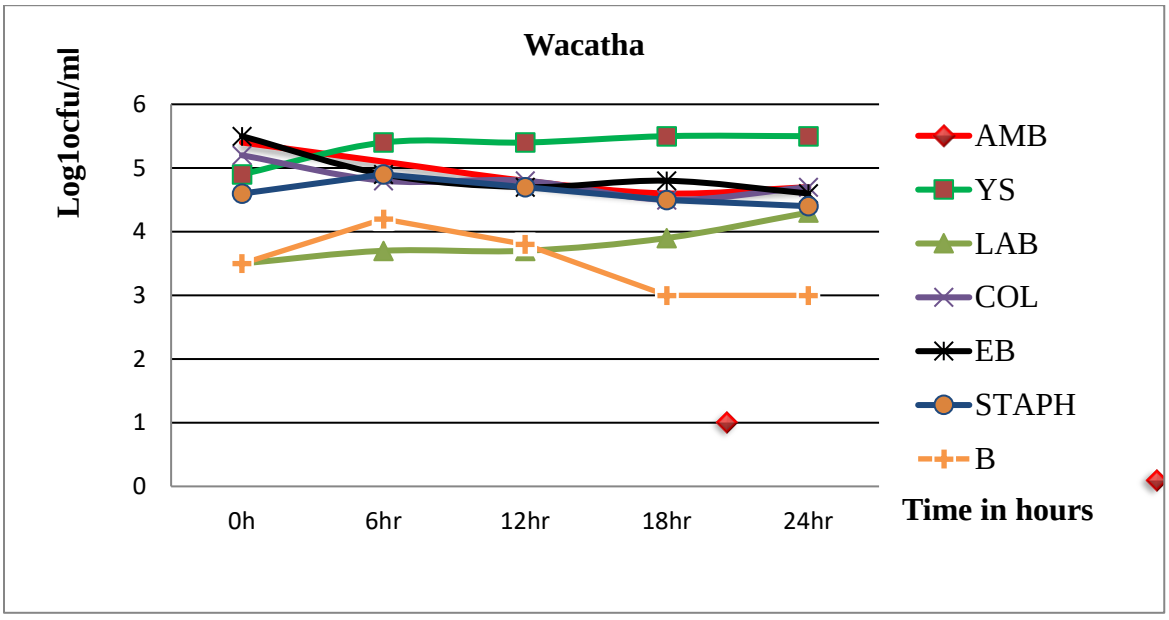

Figure 4. The count (log10 CFU/g-^{1}) of Microbial Growth under laboratory prepared Wacatha during Fermentation Time.

Where, *AMB = Aerobic mesophilic Bacteria; YS = Yeasts; Bacteria; LAB = Lactic acid bacteria; COL=Coliform; EB= Enterobacteriaceae; STAPH= Staphylococci, and B=Bacillus*

4.7 Microbial growth curve of Ogoli under laboratory

Ogoli beverage microbial growth dynamic curve fermented in laboratory sample drawn four-time after one hour's gaps 0hr, 1hr, 3hr, and 4hr shows that at zero hour *Coliform* (5.5log), *aerobic mesophilic bacteria* (5.2log), *yeasts* (5.1log), *Enterobacteriaceae* (4.8log),), *Staphylococcus* (4.4log), *bacillus* (4.2log), and *Lactic acid bacteria* (3.5log). While at one hour show that yeasts (5.4log) were dominated the fermentation followed by *Enterobacteriaceae*, (5.2log) *Aerobic mesophilic bacteria*, (5.1log), *Staphylococcus* (4.9log), *Coliform*, (4.6log) *Lactic acid bacteria* (3.7log), and *bacillus* (3.4log). At two hours fermentation show that *yeasts* (5.4log), *Enterobacteriaceae* (4.4log), *Aerobic mesophilic bacteria*, (5.1log) *Staphylococcus*, (4.7log), *Coliform* (4.7log), *Lactic acid bacteria* (3.7log), and bacillus (3.4log) and Finally at three hours showed yeasts (5.5log) were the leading followed by *Enterobacteriaceae* (4.5log), *Aerobic mesophilic bacteria* (4.4log), staphylococcus (4.4log) and *Coliform* (4.4log) were equal, *Lactic acid bacteria* (4.3log) and the last one was *Bacillus* (3log) (Figure.8).

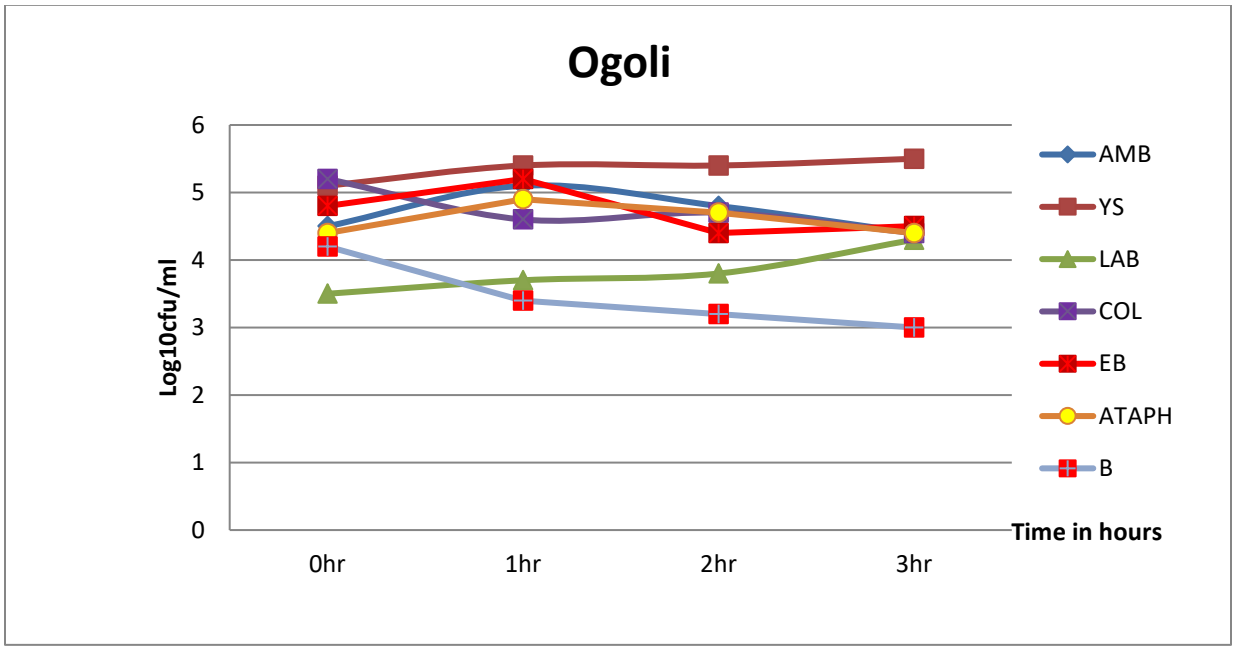

Figure 5. The count (log10CFU/ml) of Microbial Growth under laboratory prepared Ogoli during Fermentation Time.

Where, *AMB = Aerobic mesophilic Bacteria; YS=Yeasts; LAB= Lactic acid bacteria; Col=Coliforms; EB= Enterobacteriaceae; and ST= Staphylococci and B=Bacillus*

4.8 Physicochemical parameters and proximate compositions of Wacatha and Ogoli

In present study, the physicochemical parameter and proximate composition in wacatha were pH (3.84±0.24), Temperature (19.7±1.8), Titratable Acidity (1.37±0.23), Alcoholic content (3.4±0.15), Moisture content (94.3±0.4), Total ash (0.25±0. 51), Total fat (0.25±05), Total protein (0.70±0.1 5), and total carbohydrate (4.4±0.41). Whereas Ogoli physicochemical and proximate compositions were pH (3.76±0.26), Temperature (15. 6±0.34), Titratable Acidity (1.31±0.25), Alcoholic content (3.3±0.10), Moisture content (94.9±0.39), total ash content (0.20±0.11), total fat contents (0.24±0.05), Total protein (0.65±0.17) and total carbohydrate (4.5±0.61) respectively. Therefore, there are significance variation between physicochemical parameters and proximate composition analyses of wac athga and ogtoli ($P<0.05$). (Table.7 and Appendix.6).

Table 7. Physicochemical parameters and proximate compositions of Wacatha Ogoli traditional fermented beverages from Abobo district, Gambella, 2021.

Physicochemical and proximate composition	Wacatha (n=30)	Ogoli (n=30)	P -value
pH	3.84±0.24	3.76±0.26	0.000
Temperature (°C)	19.7±1.8	15. 6±0.34	0.010
Titratable acidity (g/100 ml)	1.37±0.23	1.31±0.25	0.000
Alcoholic content (g/100 ml)	3.4±0.15	3.3±0.10	0.000
Moisture content (g/100 ml)	94.3±0.4	94.9±0.39	0.024
Total ash content (g/100 ml)	0.25±0. 51	0.20±0.11	0.191
Total fat content (g/100 ml)	0. 25±050	0.24±0.05	0.000
Total protein (g/100 ml)	0.70±0.1 5	0.65±0.17	0.000
Total carbohydrate (g/100 ml)	4.4±0.41	4.5±0. 61	0.003

5. Discussion

In the present study, the sociodemographic of wacatha vendors showed that, the majority were (36.6%) range between 31-36 age. while the majority of ogoli vendors (60%) range between 37-43 ages. About (80%) of wacatha were female, while (70%) of ogoli vendors were male. Therefore, the highest majority of Ogoli male vendors might be due to hard activities related to harvesting of honey and other indigenous believe that fermentation of Ogoli made by a male, women are not allowed to approach until is ready to consume. the majority of both wacatha and ogoli vendors have used reagents for cleaning materials. Wacatha vendors' education levels, (46.7%) were in secondary, and ogoli (50%) were in primary.

In the present study, microbial count of yeast (log $CFUg^{-1}$) in Wcatha (7.2±0.3) was highest than yeast count of ogoli (7.0±0.2) that could be due to long (Twenty four hours) fermentation of wacatha then ogoli(Three hours) and second reason in the presence of a high count of yeasts in both wacatha and ogoli in present study could be due to Agiima and enkuro as they are considered as a starter culture of the fermentation. In the present study, the microbial count of aerobic mesophilic bacteria in Wacatha (7.05 log $CFUg^{-1}$) was highest than in Ogoli (6.9 log $CFUg^{-1}$) the high count of aerobic mesophilic bacteria in wacatha then in ogoli could be due to unsanitary of the vendors.

Enterobacteriaceae in Wacatha *(*4.7 log $CFUg^{-1}$) was least than Ogoli (5.6 log $CFUg^{-1}$). Present of aerobic mesophilic bacteria in both wacatha and ogoli is an indicator of Enterobacteriaceae that are an evidence of poor hygienic conditions of unsafe water used during the ready-to-consume wacatha and ogoli wash utensils. The presence of Aerobic mesophilic bacteria and Enterobacteriaceae might be due to contamination ingredients such as enkuro, Agiima and malt, materials, and processing that showed poor hygiene that could result to source of foodborne disease (Motarjemi *et al.,* 1993). In the current study, Coliform in Wacatha (6.6 log $CFUg^{-1}$) was highest than Coliform in Ogoli (6.3 log $CFUg^{-1}$). The presence of coliform in this study might be due to water used in washing utensils or directly from the hands or bodies of vendors. Of course, once introduced into the food samples and the foods left at ambient temperature for a while, the contaminating coliform would multiply to higher counts (Tomkins, 1981). The coliforms have been used as an indicator of sanitary conditions (Sousa, 2005).

In present study aerobic spore former bacteria in Wacatha (1.6 log $CFUg^{-1}$) were least than Aerobic spore former bacteria in Ogoli (2.4 log $CFUg^{-1}$) that in line with Aerobic spore former bacteria in tej samples, that ≤ 3 log $CFUg^{-1}$ according to report by Nemo and Bacha, (2020). As tej beverage has relatively share ingredients with Ogoli and wacatha in term of honey for preparation. The high count of aerobic spore former bacteria in ogoli might be due to short time fermentation than wacatha fermentation duration. Presence of Aerobic spore former bacteria in both wacatha and ogoli beverages could be from ingredients which were used for both wacatha and ogoli beverages preparation and the low count of arobic spore forming bacteria in both determined by present of low pH value and Additionally Aerobic spore former bacteria are fermentative that may contribute to fermentation but in the presence of sufficient numbers of LAB they could not contribute to fermentation as aerobic mesophilic spore forming bacteria they could not multiply at low pH (Ashenafi & Mehari, 1995).

Staphylococcus in Wacatha (5.85±0.34) was least than *Staphylococcus in* Ogoli *(*6.60±0.27). The presence of high count of staphylococcus aureus could be due to the unhygienic handling of the wacatha vendor than Ogoli vendors since Staphylococci exist in air, dust, sewage, water, and food or on food equipment and environmental surfaces additionally staphylococcus has an attachment to human different parts of the body such as the nose, hands, hair, eyes, and throat of healthy persons (Hammad, 2004). The mean counts of staphylococci cause risk of enterotoxin production as toxin production among these groups is possible after the counts exceed or equals 106 CFU mL-1 (James, 2000).

In the present study, LAB in Wacatha (5.0±0.40) was relatively the same as LAB in Ogoli (5.0±0.5). The high count of LAB in food has a significant impact in lowering the counts of pathogens since can produced antimicrobial compounds against competing flora including foodborne spoilage and pathogenic bacteria (Davidson and Hoover, 1993). High count of LAB could account for acidification of the product with extension of fermentation periods. LAB has been involved in the natural fermentation of many traditional Ethiopian fermented foods and beverages (Bahiru *et al.,* 2006). In case of the low count of Lactic Acid Bacteria might be due to ingredients used for fermentation and environmental factors According to Kirchmayr *et al.,* (2017) such as temperature and moisture content (Almeida *et al.,* 2007).

In the present study, Although there was no quality guideline set for traditional fermented beverages, According to Gilbert *et al.,* (2000). The microbial quality count standard (log CFU/ml) in both Wacatha and Ogoli beverages showed that *Aerobic mesophilic bacteria, Coliform, Staphylococcus* were (*>5logCFU/g*$^{-1}$) did not meet with the guideline set as a result the counts are under unsatisfactory since they are considered to cause illness except Aerobic spore former bacteria which are acceptable as in both wacatha and Ogoli aerobic spore forming Bacteria counts were *3logCFU/g*$^{-1}$. The presence of high counts of *Aerobic mesophilic bacteria, Coliform, Staphylococcus, enterobactereae* are might be due to ingredients, production and handling contamination and the poor hygienic conditions of the fermented beverage (Abawari, 2013). As the hands of the food handlers are the most important vehicle for the transfer of organisms from feces, nose, and skin to the food (WHO, 1989). Whereas the presence yeast and LAB in present sudy of microbial dynamic are known for significant implications in beverage, and industrially valuable products such as lactic acid, bacteriocins, and other useful metabolites (Bintsis, 2018; Mora-Villalobos *et al.,* 2020).

The present study on microbial growth dynamic of Wacatha and ogoli fermented beverage prepared under laboratory showed at early stages, the fermentation was dominated by Enterobacteriaceae, aerobic mesophilic bacteria, Coliform, Staphylococcus by microbial count (<5.5logCFUg^{-1}) while in both wacatha and ogoli fermentation, at the end of fermentation yeast dominated and Lactic acid bacteria was progressively growing as yeast and LAB could have a mechanism to survive in acidic condition. The microbial dynamic was in agreement to Nemo and Bacha, (2021) on borde microbial dynamic, that at the beginning of the fermentation, Aerobic mesophilic bacteria staphylococci, and Enterobacteriaceae initiated the fermentation process (>5 logs CFU/ml) but yeast and LAB dominated the fermentation process until the end of fermentation.

In the present study, the pH of wacatha (3.84±0.24) was highest than pH in Ogoli (3.76±0.26) that showed present study is relatively in line with report by Bahiru *et al.,* (2021), that the pH values in tej samples were between (3.07 and 4.90) respectively. Low pH value of both wacatha and ogoli beverage could be due to the production of organic compound such as Acetic acid and propionic acid produced by LAB strains according to Behera *et al.,* (2018). While the temperature in wacatha (19.7±1.8) was highest than the temperature in Ogoli (15. 6±0.34) that

showed the present study were least than the temperature in tej (22.87 ± 0.90) according to Nemo and Bach, (2020). Titratable Acidity in Wacatha (1.37±0.23) was highest than titratable acidity in Ogoli (1.31±0.25) that showed titratable acidity in the present study was greater than titratable acidity in tej which between (0.34 to 0.6) according to Bahiru *et al.,* (2006). In the present study, the alcoholic content of Wacatha (3.4±0.15) was highest than Alcoholic content in Ogoli (3.3±0.10). Low alcoholic content in the present study might be due to the short fermentation time of the wacatha beverages which is twenty four (24) hours and Ogoli three (3) hours . As yeast is responsible for the production of alcohol with the availability of substrate (Wassie, 2016).

In present study, Moisture content in Wacatha (94.3±0.4) was least than in Ogoli (94.9±0.39) that showed present study moisture content least than in tej (95.78 ± 1.21) by Nemo and Bacha, (2020). Therefore, an increase in the moisture content can be recognized by the addition of water to the substrate before fermentation (Ekundayo *et al*., 2013). While high moisture content and high pH of a beverage favor growth of many types of microorganisms (Holzapfel *et al.,* 2001). Total ash content in wacatha (0.25±0. 51) was highest than ash content in ogoli (0.20±0.11) that showed present study has least ash content than in booka (0.82+0.06) while total fat in Wacatha (0.25±050) was highest than in Ogoli (0.24±0.05) which present study least than total fat in Booka (1.17 to 1.81) According to Binitu *et al.,* (2018). Total protein in wacatha (0.70±0.1 5) was highest than in Ogoli (0.65±0.17) whereas the total carbohydrate in wacatha (4.4±0.41) was least than (4.5±0. 61) in ogoli that showed, the present study is in line with Ashenafi. (1997) study on tej that the range of total protein was (0.33–4.66) and the range of total carbohydrates was (1.49–3.79). In the present study, proximate compositions showed a significance variation between each beverage from collected samples ($p<0.05$).

Microorganisms involves in traditional fermented beverages are depend on the physicochemical properties and proximate composition according to Cousin *et al*., (2017), that have an impact on the safety and quality of beverages: reduce the microbial load and improve organoleptic properties (Holzapfel *et al.,* 2001). The low pH in the present study in both Wacatha and Ogoli beverages are an indicator of lactic acid production of organic acids. In addition, Even though yeast counts are higher than LAB count they are important in producing secondary metabolites that lowering the pH by producing organic acids and alcohols. However, the short fermentation

time of Wacatha and Ogoli caused low alcoholic as more alcohol production required extended fermentation time with an abundant substrate (Walker & Stewart, 2016).

6. Conclusions and Recommendations

6.1 Conclusions

The following conclusions were made according to the finding of the study.

- In the present study, the sociodemographic of the wacatha vendors showed that females were higher than males whereas in Ogoli vendors males were the majority.
- The microbial count of aerobic mesophilic bacteria, coliforms, staphylococcus, Enterobacteriaceae were found in high count that considered as a risk for consumers.
- Microbial dynamics from both beverages at the beginning of fermentation most probably were dominated by poor quality and spoilage indicator microbes such as Enterobacteriaceae, Coliform and Aerobic mesophilic bacteria but at the end were dominated by yeast.
- The results of microbiological, Physico-chemical, and nutritional value in *Wacatha* and *Ogoli* were used to improve the efficiency of the beverarages process.

6.2. Recommendations

- In this finding isolation of pathogens, staphylococcus aureus, and E. coli in homemade Wacatha and Ogoli beverage prepared for consumers at home comprises a likely potential hazard to human health
- In this study the presence of a high count of Coliform and Aerobic mesophilic bacteria is a poor quality indicator, ingredients as well as materials therefore proper utensil during production is mandatory.
- The isolation of Enterobacteriaceae in ready-to-use Wacatha and Ogoli is a good indicator of unhygienic producing and handling contamination. Therefore, producers should improve Wacatha and Ogoli venders quality and safety.
- Furth
- More investigation on the result of proximate composition of both wacatha and ogoli beverages would be mandatory.
- Health sectors, municipalities, and Nongovernmental organizations should work together to improve the sanitary facilities of the community and in turn protect the health of the consumers to reduce the public and economic burden caused due to foodborne diseases.
- Finally, this study may serve as a foundation for further studies on the process of optimization of *Wacatha and Ogoli* and its production. Therefore, it requires further study on the microbiology and handling process of *Wacatha and Ogoli beverage*

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Appendices

Jimma University

College of Natural Sciences

Department of Biology (Applied Microbiology)

Interview designed for Wacatha and Ogoli beverage producer from Abobo district, Gambella western Ethiopia

Dear respondent

The objective of this questionnaire was used to interview Wacatha and Ogoli producers randomly to gather applicable information related to fermentation to document the microbiology and physicochemical properties of locally fermented beverages in the Anywaa zone Abobo district, Gambella, western Ethiopia. The truth and honesty of the information you provide were determined by the relevance of data generated and the significances of the decision made on the collected data. Any information obtained from respondents was kept confidential and used only for scientific purposes and the improvement of Wacatha and Ogoli beverage production and safety. So, you are selected randomly as a respondent in this survey just owing to chance and have nothing to do with your identity. You are kindly requested to answer every question.

Thank you in advance!

Part- I: Socio-demographic characteristics of Wacatha and Ogoli vendors

Instruction: Choose from among the alternatives for choice cases and underline the letter of your choice or respond to an open-ended questionnaire by writing on the space provided.

1. Sex: a) Male b) Female

2. Age in years ----------

3. What is your level of educations?

A) Illiterate B) literate C) Primary D) secondary E) if others, specify.....

4. How long have you been fermenting? A) >5 B) 6-10 C) 11-15 D) 16-20

II Background of Wacatha and Ogoli beverages fermentation

1. Are you familiar with wacatha and Ogoli fermentation? A) Yes B) No

2. Do you know Wacatha and Ogoli? A) Yes B) No

3. If you have knowledge about Wacatha and Ogoli from where did you get it?

A) Training B) Family C) Relatives D) I don't know! Why.....

III Sanitation habit of Wacatha and Ogoli beverages vendors

1. Where did you store your fermented Wacatha and Ogoli beverages?

A) refrigerator B) with a bucket in-floor C) shelves

2. Do you clean the materials? A) Yes B) no

3. Do you use reagents to clean materials and place? A) Yes B) No

4. What reagent do you use to clean material? A) Water and Omo B) only water C) water and soap D) If others specify.....

5. Do you have vending and selling place? A) Yes B) No

7. Have you used to wearing a coat? A) Yes B) No

Appendix.1 Socio-demographic characteristics of Wacatha and Ogoli vendors

Parameters	Character	Wacatha respondents (N=30)	Wacatha (%)	Ogoli respondents (%)	Ogoli (%)
Age	25-30	3	10	3	10
	31-36	11	36.6	7	23.4
	37-43	12	40	18	60
	44- 50	4	13.3	2	6.6
Sex	Male	6	20.0	21	70.0
	Female	24	80.0	9	30.0
Level of your Education	Illiterate	5	16.7	6	20.0
	Primary	10	33.3	15	50.0
	Secondary	14	46.7	8	26.7
	Degree	1	3.3	1	3.3
Experience in vending beverage (years)	< 5	4	13.3	3	9.9
	6-10	6	20.0	4	13.1
	11-15	13	43.3	10	33.3
	16-20	7	23.4	13	43.4

Appendix.2 Background knowledge of Wacatha and Ogoli beverages vendors

Questions	Respondents	Frequency Of Wacatha	Wacatha (%)	Frequency of Ogoli	Ogoli (%)
Familiarity with Ogoli fermentation	Yes	30.0	100.0	30	100.0
	No	0.00	0.00	0.0	0.00
Do you know Ogoli	Yes	30	100.0	30	100.0
	No	0.0	0.00	0.0	0.00
where did you get knowledge about Ogoli	Family	16	53.3	7	23.3
	Relative	14	46.7	23	76.7

Appendix.3 Sanitation habit of the Wacatha and Ogoli beverages vendors

Characters	Respondents	Frequency Of Wacatha	Wacatha (%)	Frequency of Ogoli	Ogoli (%)
Where do you store	Bucket	14	46.7	4	13.3
	Shelves	16	53.3	26	86.7
Do you clean material and place	Yes	0.0	100.0	30	100.0
	No	0.0	0.00	0.0	0.00
Do you use	No	2	6.7	9	30.0

reagent	Yes	28	93.3	21	70.0
What reagent do you use	Only water	2	6.7	9	30.0
	Omo	11	36.7	11	36.7
	Soap	11	36.7	7	23.3
	Other	6	20.0	3	10.0
Do you have vending	No	10	33.3	6	20.0
	Yes	20	66.7	24	80.0
Do you wear a coat	No	28	93.3	00	0.00
	Yes	2	6.7	30	100.0

Appendix.4 Wacatha different microbial mean count (log10CFU/ml ± SD) descriptive

	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
YS	30	6.20	7.90	7.1733	.06914	.37868
AMB	30	6.60	7.50	7.0540	.04442	.24330
COL	30	5.56	7.20	6.6700	.07910	.37103
STAPH	30	4.90	6.80	5.8545	.07259	.34049
LAB	30	4.49	5.60	5.0243	.06557	.35912
ENTERO	22	4.00	6.70	4.6773	.11414	.53535
ASFB	23	2.40	2.80	2.5826	.02787	.13366

Appendix.5 Ogoli different microbial mean count (log10CFU/ml ± SD) descriptive

Ogoli different microbial mean count (log10CFU/ml ± SD) descriptive						
Microbes	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
YS	30	6.40	7.40	7.0800	.03877	.21238
AMB	30	6.30	7.30	6.9400	.03763	.20611
COL	30	5.50	7.07	6.6245	.08827	.39473
STAPH	30	6.08	6.90	6.6090	.06153	.27518
EB	30	5.30	5.80	5.5950	.03283	.14681
LAB	30	4.50	5.70	5.0223	.08373	.45862
ASFB	23	2.40	2.70	2.4717	.01261	.06909

Appendix.6 Wacatha physiochemical properties

Descriptive Statistics

	N	Minimum	Maximum	Sum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
MC	30	93.70	95.70	5663.66	94.3943	.05210	.40359	.163
T^{O}	30	10.80	22.50	1181.15	19.6858	.23231	1.79947	3.238
CHO	30	3.18	5.23	263.48	4.3913	.05321	.41219	.170
pH	30	3.44	4.10	230.86	3.8477	.03110	.24092	.058
Alcohol	30	3.10	3.72	202.06	3.3677	.02007	.15546	.024
Titratable	30	.96	1.89	82.60	1.3767	.03035	.23511	.055
CP	30	.33	.94	42.22	.7037	.02018	.15630	.024
ASH	30	.20	.33	15.48	.2580	.00661	.05122	.003
FAT	30	.20	.33	15.16	.2527	.00647	.05011	.003

Appendix.7 Wacatha physiochemical properties from ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MC	Between Groups	6.429	29	.222	2.091	.024
	Within Groups	3.181	30	.106		
	Total	9.610	59			
CP	Between Groups	1.262	29	.044	7.256	.000
	Within Groups	.180	30	.006		
	Total	1.441	59			
FAT	Between Groups	.124	29	.004	5.235	.000
	Within Groups	.024	30	.001		
	Total	.148	59			
ASH	Between Groups	.119	29	.004	3.448	.001
	Within Groups	.036	30	.001		
	Total	.155	59			
CHO	Between Groups	7.220	29	.249	2.663	.005
	Within Groups	2.805	30	.093		
	Total	10.024	59			
TEMPERATURE	Between Groups	129.334	29	4.460	2.168	.019
	Within Groups	61.714	30	2.057		
	Total	191.048	59			
ALCOHOL	Between Groups	1.358	29	.047	20.684	.000
	Within Groups	.068	30	.002		
	Total	1.426	59			
TITRATION	Between Groups	2.949	29	.102	9.782	.000
	Within Groups	.312	30	.010		
	Total	3.261	59			
pH	Between Groups	3.409	29	.118	221.535	.000
	Within Groups	.016	30	.001		
	Total	3.424	59			

Appendix.8 Ogoli physiochemical properties Descriptive Statistics

Descriptive Statistics

	N	Minimum	Maximum	Sum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
MC	30	94.20	95.80	5692.00	94.8667	.05068	.39259	.154
T^O	30	15.10	16.30	937.00	15.6167	.04479	.34698	.120
CHO	30	3.33	5.54	272.26	4.5377	.07990	.61892	.383
pH	30	3.25	3.97	225.75	3.7625	.03449	.26714	.071
Alcohol	30	3.10	3.53	198.44	3.3073	.01369	.10607	.011
Titratable	30	.88	1.89	78.75	1.3125	.03313	.25662	.066
CP	30	.38	1.02	39.55	.6592	.02289	.17734	.031
FAT	30	.10	.33	14.88	.2480	.00685	.05307	.003
ASH	30	.10	.90	12.50	.2083	.01432	.11095	.012

Appendix.9 Ogoli physiochemical properties Descriptive Statistics ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MC	Between Groups	7.277	29	.251	4.144	.000
	Within Groups	1.817	30	.061		
	Total	9.093	59			
CP	Between Groups	1.677	29	.058	9.715	.000
	Within Groups	.179	30	.006		
	Total	1.855	59			
FAT	Between Groups	.137	29	.005	4.944	.000
	Within Groups	.029	30	.001		
	Total	.166	59			
ASH	Between Groups	.378	29	.013	1.123	.377
	Within Groups	.348	30	.012		
	Total	.726	59			
CHO	Between Groups	18.613	29	.642	4.828	.000
	Within Groups	3.988	30	.133		
	Total	22.600	59			
TEMPERATURE	Between Groups	5.327	29	.184	3.102	.001
	Within Groups	1.777	30	.059		
	Total	7.103	59			
ALCOHOL	Between Groups	.562	29	.019	5.703	.000
	Within Groups	.102	30	.003		
	Total	.664	59			
TITRATION	Between Groups	2.990	29	.103	3.454	.001
	Within Groups	.895	30	.030		
	Total	3.885	59			
pH	Between Groups	4.180	29	.144	144.879	.000
	Within Groups	.030	30	.001		
	Total	4.210	59			

Appendix 10. John's bacterial identification for Wacatha and Ogoli

Gram staining	+	+	+	+	+	+	+
Shape	**Rod**	**cocci**	**cocci**	**Cocci**	**cocci**	**cocci**	**rod**
Motility test	-	-	-	-	-	+	+
Endospore	-	-	-	-	-	+	_
Catalase	-	-	-	-	-	+	+
KOH	-	-	-	-	-	-	-
TSIA Test	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-
Citrate	+	+				+	+
Fermentation of Acid							
Glucose	+	+	+	-	-	-	-
Sucrose	+	+	+	-	-	-	-
Maltose	+	+	+	-	-	-	-
Acid pH	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Nacl2							
4%	+	+	+	+	+	+	+
6%	+/-	+/-	+/-	+/-	+/-	+/-	+/-
7%	-	-	-	-	-	-	-
Lactobacillus	**X**						
Enterococcus		**X**					
Lactococcus			**x**				
Leuconostoc				**X**			
Staphylococcus					**X**		
Bacillus						**x**	
Pseudomonas							**x**

Appendix .10 John's bacterial identification

Gram staining	+	-	-	-	-	-	-	-	-	-	-
Shape	R	C	C	C	C	C	C	C	C	C	R
Motility test	-	-	-	-	+	+	-	-	-	+	-
Endospore	-	-	-	-	-	-	-	-	-	+	-
Catalase	+	+	+	+	+	+	-	+	+	+	+
KOH	-	-	-	-	-	-	-	-	-	-	
TSIA Test					$A^{+}K^{-}$ $G^{+}H_2S$	$A^{+}K^{-}$ $G^{+}H_2S$				$A^{-}K^{+}G^{-}$	$H2S^{+}$
Urease	+	+	+	+	+	+	+	+	+	+	±
Acid production											
Glucose	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+
Saccharomyces	**X**										
Pediococcus		**X**									
Escherichia			**X**	**X**							
Citrobacter					**X**						
Enterobacter						**X**					
Streptococcus							**X**				
Micrococcus								**X**			
Providencia									**X**		
Proteus										**X**	
Klebsiella											**X**

Declaration

I declare that this thesis entitled “Microbiology and proximate composition analysis of some traditionally prepared beverage in Abobo district, Gambella, western Ethiopia", submitted for the Award of Degree of Masters of Science in Applied Microbiology 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 another university.

Lero Obang Okoti _____ _____

Signature Date

The work has been done under the supervision

Name Signature Date

1. Shiferaw Demissie (MSc) _____ _____

2. Lata Lechisa (MSc) _____ _____