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ANALYSIS OF PHYSICOCHEMICAL PARAMETERS AND MICROBIAL LOADS OF *TELLA* FROM JIMMA CITY, ETHIOPIA

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ANALYSIS OF PHYSICOCHEMICAL PARAMETERS AND MICROBIAL LOADS OF *TELLA* FROM JIMMA CITY, ETHIOPIA

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Title**:** Analysis of physicochemical parameters and microbial loads of *tella* from Jimma city, Ethiopia

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List of Abbreviations

ABV: Alcohol by Volume

ANOVA: Analysis of Variance

AMB: Anaerobic Mesophilic Bacteria

CFU: Colony Forming Unit

LAB: Lactic Acids Bacteria

MRS: de Man Rogosa Sharpe

PCA: Plate Count Agar

TA: Titrable Acidity

VRBA: Violet Red Bile Agar

YGC: Yeast extracts Glucose Chloraphenicol

EBC: European Brewery Convention

Abstract

Tella is home processed traditional fermented alcoholic beverage commonly consumed in Ethiopia. In depth study of its physicochemical parameters including microbiology is vital for safety of the community who drinks *tella*. Therefore, the aim of this study was, to analyze fermented *tella* quality in terms of physicochemical parameters (alcohol, CO_2, titratable acidity (TA) contents, pH, refractive index and microbial loads) of *tella* from Jimma city. To study the parameters, 19 total *tella* samples were purposively collected from three *kebeles* of Jimma city such as *Kochi*, *Matrik Sefer* and *Merkato* in the morning. Standard analytical methods were used for analysis of physicochemical and microbial load of *tella*. The analysis, of *tella* from sampling sites of (*Kochi*, *Matrik Sefer* and *Merkato*), showed Anaerobic Mesophilic Bacteria (AMB): 4.28 ± 0.03, 5.5 ± 0.04, 4.64 ± 0.03 log CFU/mL, lactic acid bacteria (LAB): 8.01 ± 0.14, 6.65 ± 0.03, 7.24 ± 0.04 log CFU/mL yeast: 6.28 ± 0.04, 5.09 ± 0.01, 6.74 ± 0.04) log CFU/mL and Enterobacteriaceae (EB): 1.1 ± 0.01, 2.02 ± 0.01, 1.14 ± 0.01 log CFU/mL, respectively. Obtained results showed that, there was significance difference ($p < 0.05$), among house of *tella* venders and samples sites in microbial load and physicochemical properties. From the study, the microbial load and physicochemical analysis were within the recommended ranges, except some of *Kochi* and *Merkato tella* has pH values below the EBC recommended. The result revealed that *Kochi tella* has significantly higher in microbial load, TA and CO_2 content than *Matrik sefer* and *Merkato tella*. In this study, microbial loads were found in the tolerable standard below 10 CFU/mL according to EBC standard. Thus, it is recommended that brewing of *tella* should be carried out in aseptic conditions in order to avoid risks of having pathogenic bacteria.

Key words: *Tella, physicochemical parameters, microbial loads.*

1. Introduction

1.1 *Tella*

The traditional beverage preparation is predominantly a household phenomenon in Ethiopia. The traditional beverages industry in the country is not well developed. Traditional alcoholic and non-alcoholic beverages like, *tella*, *tej*, *birz*, *borde*, *korefe* and *areke* are indigenous to Ethiopia. [1]. In Ethiopia, the traditional cereal based alcoholic fermented beverages are still prevailing in both rural and urban communities. Cereals serves as sources of diet and such diet are major sources of energy, protein, B vitamins and minerals for human beings. Beverages are liquid foods that provide nutrient. Like cereals, they also provide energy for human beings [2].

Among various Ethiopian traditional cereal based fermented alcoholic beverages, *tella* is the most popular. Is an indigenous, a home processed traditional fermented alcoholic beverage in Ethiopia. It has many varieties in the various regions and is made with diverse cereals such as barley, wheat, maize, millet, sorghum, and teff [3]. *Tella* is not on the market as a commercial product in Ethiopia but it produced and sold domestically. A household with standing sticks with paper cups or bags in the front indicating they are selling their homemade *tella* is a common sight on countryside roads. Its production depends on the naturally present micro flora in the substrates and equipment used as well as the environment of the households. Due to the addition of bread and use of a fermentation vessel which has been smoked over dried olive wood, *tella* may have a smoky flavor.

Tella and its products are used as human diet and source of calories. The primitive beverages provide not only calories but also vitamins B, due to residues of the substrates, the fermenting yeasts and other microorganisms [4, 5]. The quality of *tella* was variable from local to local, from individual to individual. Even within the same individual, the quality was variable from time to time. Because *tella* has not been standardized and modernized. The quality of *tella* is a term with a very broad meaning. Quality *tella* means, *tella* which is free from pathogenic

bacteria and harmful toxic substances, free from sediment and extraneous substances, of good flavor, with normal composition, adequate in keeping quality and low in bacterial counts. However, *tella* may be contaminated by various environmental pollutants from agricultural and hygienic practices. Fresh *tella* can be easily deteriorates to become unsuitable for processing and human consumption. This ultimately leads to the stage that the consumer becomes victim of diseases like mouth cancer, diarrhea or several days vomiting. Physicochemical analysis is an important tool to monitor the quality of *tella* beverages. Physicochemical parameters of *tella* product can be affected by adulteration, which is done either for financial gain, or lack proper hygienic conditions during processing and storing [6].

Microorganisms were involved in *tella* fermentation, because of its nutritious nature that makes *tella* to be ideal for microbial growth. They produce desirable flavor and physical changes during *tella* fermentation. They keep spontaneity of fermentation and maturation of *tella* beverages [4]. Microorganisms in *tella* can originate from different sources such as ingredients, air, equipment, procedures' and water used. Total bacterial counting has become one of the accepted criteria for grading *tella* intended for consumption and processing of *tella* products. In Ethiopia *tella* is produced traditionally by individuals. In Jimma city, although there are *tella* vendors, the quality of *tella* there is not known. People also prefer some venders, suspecting that they supply quality *tella* than the others. Therefore, investigation of the physicochemical properties and microbial load of *tella* of Jimma city is important to identify where *tella* vendors supply similar *tella* quality to the community or not.

1.2 Statement of the problem

A traditional alcohol drinks produced for home consumption or limited local trade and unregistered is found in virtually every country around the world. Ethiopia is one of the country in which traditional alcohol that reflects the local drinking culture and occupies a particular place within a given society. Majority of physicochemical and biological parameters of Ethiopian traditional fermented beverages have not been documented. From these beverages, *tella* was the most popular unrecorded alcohol in Ethiopia. It can be used largely for personal consumption, and for sale.

There were many researches on Ethiopian *tella*, Yohannes, et al [6], done on preparation and physicochemical analysis of parameters including refractive index, specific gravity, alcohol contents and pH of traditional alcoholic beverages from Jimma, but not include titratable acidity, CO_2 contents and microbial loads of *tella*. Berhanu, A [16], explained about microbial profile and role of *gesho* in *tella*, but not includes alcohol, CO_2, and titratable acidity contents of *tella*. Getachew Tafere and Berihu Tekluu [32], explained about specific gravity, moisture, color, real degree fermentation, original extracts, real extract, alcohol and CO_2 contents of *tella*, but not include titratable acidity, microbial loads and pH of *tella*. Debela Taddele [10], done on production, optimization and characterization of *tella*. However, physicochemical parameters like CO_2, titratable acidity contents and microbial load of *tella* were not considered. But physicochemical parameters like pH, titratable acidity, CO_2 and microbial loads for *tella* and its quality comparison with EBC and Beddelle beer factory inquire detail investigation and need extensive assessments from Jimma city. Therefore, the aim of this study was, to analyze physicochemical parameters (alcohol contents, CO_2 contents, titratable acidity, pH, refractive index and microbiological loads of *tella* of Jimma city *tella* venders.

1.3 Objectives

1.3.1 General objective

The main objective of the study was to investigate the physicochemical parameters and microbial loads of *tella* from Jimma city.

1.3.2 Specific objectives

- To determine physicochemical properties such as alcohol contents, pH, CO_2 contents, and titratable acidity of fermented *tella* from Jimma city.
- To assess the microbial load such as lactic acid bacteria, coli form bacteria, yeast and anaerobic mesophilic bacteria
- To evaluate the quality of tella with EBC (European brewery convention) and Beddelle beer factory quality standard.

1.4 Significance of the study

The findings of the study could be used as background information about physicochemical properties and microbial load of *tella* of Jimma city. It also serves as a reference for further study who wants to conduct a research on *tella* and traditional alcoholic beverages.

2. Review literature

2.1 Traditional fermented Alcoholic beverages

A number of food fermentation processes, including those of dairy products, sausages, pickles, sauerkraut, alcoholic beverages and bread have been extensively investigated and documented. Many other foods, which are prepared by the action of diverse microorganisms on plant materials, are little known outside their native countries. On the basis of the important role played by the traditional African fermented beverages, the consumers tend to recognize these beverages as types of food rather than just beverages. Moreover, traditional fermentation processes are increasingly attracting the attention of scientists and policy makers as a vital part of food security strategies [7]. New opportunities provided by biotechnology are opening up possibilities to improve or upgrade traditional small-scale processes and make better use of agricultural products. Most of the customs and rituals involving the Ethiopian traditional fermented beverages are still prevailing today in urban areas, village communities and rural households.

Tella is an indigenous, a home processed traditional fermented alcoholic beverage in Ethiopia. It can be prepared from different ingredients such as barley, wheat, maize, millet, sorghum, teff or other cereals. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia. It is well known as local beer since it is malt based beverage like that of commercial beer. Unfiltered tella is types of *tella* that can be consumed directly without storing in closed container. The alcohol content of unfiltered *tella* is usually around 2–4% Volume percent [8]. With regard to the substrate, there is no as such basic difference between *tella* and beer. But, the difference between *tella* and beer is that there is no yeast added in step of *tella* production. Its production depends on the naturally present microflora in the substrates, utensils, and equipment used, and the environment of the households [9].

2.1.3 Preparation of *tella*

Soaking barley in water (to preparing the sugar present in barley for enzymatic or microbial action)→ germination or malting**(**activated by water and oxygen, the root embryo of the barleycorn secretes a plant hormone called gibberellic acid, which initiates the synthesis of α-

amylase)→ kilning**(**curing at higher temperatures promotes a reaction between amino acids and sugars to form melanoids, which give both color and flavor to malt) → milling **(**grinding solid malt into powder) →mashing(malt and *gesho* is mixed in water using mixer) → fermented *tella* [6].

2.1.4 Alcoholic fermentation

Alcoholic fermentation occurs in yeast species that have metabolic pathways for converting pyruvic acid to ethanol. Alcoholic fermentation is the conversion of the principal sugars glucose and fructose, to ethanol and carbon dioxide that conducted by yeasts of the genus *Saccharomyces cerevisiae (top* fermentation used for the production of stout and ale, using its strains) and *Saccharomyces uvarum (*bottom fermentation used for lager production. Species of *Saccharomyces* are ambient in the environment, and are present on the skins of fruits such as ripe grapes [10, 11]. Sugar concentration is reported to affect the yield of alcohol produced. Nearly equal weights of ethanol and carbondioxid are produced and the carbon dioxide flushes out residual oxygen and maintains the fermentation anaerobic. The yeasts multiply and ferment rapidly and other microorganisms most of which are aerobic cannot compete [12]. In post fermentation, alcohol run from the fermentor is not ready for drinking because it contains suspended particles and is therefore hazy, lack sufficient carbonation, the flavor is not fully matured, it is physically and microbiologically unstable and flavor and color may need to be adjusted.

2.1.5 Fermentation process of *tella*

Fermentation is metabolic process that produces chemical changes in organic substrates through action of enzymes. Fermentation process enhances the nutritional quality of raw ingredient by improving the digestibility of nutrients and inactivating anti-nutritional factors and also improves the acceptability of beverages by destroying undesirable flavor of the raw ingredients. It is a metabolic process in which an organism converts a carbohydrate, such as starch or a sugar, into an alcohol and carbondioxid.

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2$$

(Sugar) (Ethanol) (Carbondioxid)

The biochemical changes, the microorganisms involved in the fermentation and those which bring about desirable and undesirable changes in the process of *tella* making are described. According to the report [10], the fermentation process of *tella* is divided into four phases. At the first stage, dried *gesho* leaves are soaked in water for 72 h. This stage is initiates for extraction of flavor, aroma and antibacterial from *gesho*. It is the stage before microbial start fermentation. The second stage starts by mixing malt (*bikil*) and water for 12h. This stage is the first step for real fermentation. At the third stage, unleavened bread pieces of (*kita*) and water are mixed for 48 h. The semisolid mixture is formed. The fourth stage, enkuro and water are mixed and left for 72 h. Then container is filled with 1:3 ratio of slurry and water are mixed thoroughly. The container is then sealed with mud to create an anaerobic condition and left for 48 h or more. After end of fermentation foam is formed. The formation of foam indicates that *tella* beverage is ready to for consumptions. *Tella* is consumed directly or after filtration.

2.1.6 Physicochemical parameters of *tella*

Safety consideration of Ethiopian foods and beverages has shown the possibility of isolating some food-borne pathogens from some fermented product. However, there is no scientifically documented information both on the microbiology and safety of *tella* fermentation. The quality of *tella* is variable from local to local, from individual to individual, even within the same individual; the quality varies from time to time. Its quality of may be deteriorated and affected by contaminants such as fungi and different types of bacteria. The spoilage reduced useful life of *tella* as a result of microbial contamination introduced into the wort by the fermenting wort. To guarantee the consistency of product quality, at different stages of *tella* production process should be monitored for the presence of spoilage microorganisms as that of beer. The physical and chemical properties of a *tella* body are characteristic of alcohol, titratable acidity, carbondioxid contents, pH and microbial loads [13].

2.1.7 Alcoholic contents

The alcohol in *tella* is a direct result of the yeast eating sugar. As the yeast consumes the sugar in the worth, it creates alcohol and carbondioxid. The carbondioxid floats up and out of *tella* while the alcohol stays behind and turns *tella* boozy. Alcohol level of *tella* was affected by fermentation time difference. The mean alcoholic content of Ethiopian traditional beverage

varies from report to report. For example, *tella* collected from Debre Berhan, Ataye and Addis Ababa has alcohol content of 2.4-3.3%, 2.1-2.7% and 1.6-2.8%, respectively [14, 15].

The main active ingredient of *tella* is alcohol and therefore, the effects of alcohol apply to *tella*. While moderate alcohol consumption can be part of a healthy lifestyle, but alcohol isn't generally considered healthy. Part of its mixed reputation comes from both the short- and long-term effects it has on your body and your health, from your brain, to your blood sugar, to your liver. Alcohol dries the mouth because bacteria from the tooth's surface, gum disease, tooth decay, and mouth sores are all much more likely for heavy drinkers, and alcohol abuse is the second most common risk factor for oral cancer and acids from citrus attack teeth. Alcohol consumed largely can affect heart and lungs, develop cancer in the mouth, throat, esophagus, colon, or liver, high blood pressure, irregular heartbeat , difficulty pumping blood through the body, stroke, heart attack, heart disease, heart failure. Therefore, checking alcohol contents were needed [7, 15, 16].

2.1.8 Refractive index

The refractive index or index of refraction of materials is a dimensionless that describes how light propagates through that medium. For example, the refractive index of distilled water is 1.333, meaning that light travels 1.333 times slower in water than in the vacuum. The refractive index determines how much the path of light is bent, or refracted, when entering a material. *Tella* that contain large refractive index have large number of suspended solids, unfermented sugar, form small alcohol and titratable acidity (TA). But *tella* which fermented well have closed to distilled water refractive indexes [6].

2.1.9 Bitterness of *tella*

Tastes of *tella*, were bitterness, sweet, and sour which similar to the description of the typical beer taste. In order to control the formation of the very sour taste due to the succeeding bacteria during the *tella* fermentation, small parts of the *tella* are removed and are tasted. People add the ash of burnt wood to neutralize the excessive acid. The bitterness of *tella* is from *gesho* [17]

Leaves of *gesho* have its own role in brewing; it gives a bitter flavor and antibacterial effects for the shelf-life. In Ethiopia, *gesho* (*Rhamnus prinoides*) is particularly used to provide a special

aroma and flavor. *Gesho* may have antibacterial effect against some groups of bacteria. The bitterness of *tella* is directly related to the amount of *gesho* added during brewing. It is assumed that *gesho* (*Rhamnus prinoides*) maintains acidic and pH during *tella* fermentation so as to modify the nature of the mash and inhibits the growth of undesirable microorganism [17, 18]. So *tella* with more gesho is more preferable.

2.1.10 Microbiological quality of *tella*

Microbial quality of *tella* refers to the cleanness of *tella*. The microbial content of *tella* is a major feature in determining its quality. It shows the hygienic level exercised during *tella* production and handling, that is cleanliness of the *tella* utensils, condition of storage, water used and manner of transport [9, 19]. Contamination of *tella* with high levels of spoilage bacteria is usually unsuitable for further processing, since it does not meet the consumer's expectations in terms of health (nutritional value), safety (hygienic quality) and satisfaction (sensory attributes).

The quality of *tella* may be deteriorated and affected by contaminants such as fungi and different types of bacteria. To guarantee the consistency of product quality, the different stages of *tella* production should be monitored for the presence of spoilage microorganisms as that of beer [20, 21]. Contamination of beverages products can result in many health problems ranging from mild bloating and gas to serious incidents of beverage poisoning and dehydration. Unsafe and hygienic of *tella* ingredients causes serious outbreaks of beverages borne illness [22]. As a result, total bacterial counting has become one of the accepted criteria for grading *tella* intended for consumption and processing of *tella* products. Lack of knowledge about clean *tella* production, use of unclean *tella* equipment and lack of potable water for cleaning purposes were some of the factors which contributed to the poor hygienic quality of *tella*. *Tella* is an important vehicle for transmission of pathogenic microorganisms to human beings unless it is produced and handled under good hygienic conditions. Thus, hygienic production of *tella* has to get attention in order to provide more and better quality *tella* for the general public [9, 19].

The detection of bacteria, pathogens and high microbial count in *tella* are major factors in determining its quality [23].

2.1.11 pH

The pH or the hydrogen ion concentration of *tella* gives a measure of the acidity of *tella*. In normal *tella*, the pH ranges from 4-5. The pH value can be lower than 4 due to development of acidity even though *tella* has normal acidity range of 0.16- 0.8%. The pH value below 4 indicates increased acidity of *tella* due to bacterial multiplication [13]. The pH of *tella* changes over time. *Tella* goes sour, it becomes more acidic and the pH gets lower. This occurs as bacteria in *tella* convert the ethanol to acetic acids. pH and oxygen are usually considered as the main factors influencing the organoleptic stability of *tella* [23].

2.1.12 Titratable acidity (TA)

The sum of natural acidity and developed acidity is known as titratable acidity. It refers the total concentration of free protons and undissociated acids in a solution that can react with a strong base (NaOH) and be neutralized. Organic acids are both naturally present in foods and beverages during fermentation or which are added to beverages during fermentation processing, have been used for beverages preservation. The most commonly organic acids include citric, succinic, malic, tartaric, benzoic, lactic and acetic acids. They do not dissociate completely [24].

Fresh *tella*, however, does not contain any appreciable amount of lactic acid and therefore an increase in acidity is a rough measure of its age and bacterial activity. Titratable acidity (TA) is a rapid test indicating *tella* quality and provides an indirect measure of the acid content in *tella*. During the course of *tella* making and in the finished *tella*, acetic, butyric, and lactic and succinic acids can play significant role. Most of the acids involved with *tella* acetic acid and other organic acids which are volatile and can contribute to the *tella* fault known as volatile acidity. Preferred total acidity levels of beverages are 4 – 8 g/L. High level of acidity in *tella* cause dental erosion. The acidity of water used in baking affects the acidity of *tella* product. Generally, as *tella* acid content increases, TA values increase [25, 26].

2.1.13 Carbondioxid content

Carbondioxid is formed during fermentation and is present in fermenting tans that contained beverages. Adding CO_2 to food packaging can considerably extend the storage and shelf life [4]. We cannot ignore its importance when considering process safety, or product quality. CO_2 gas is used in the carbonation of soft drinks, beers and *tella*, because many studies have demonstrated that CO_2 prevents fungal and bacterial growth as well as used as foaming agent, coloring and for flavor. But very high concentrations of CO_2 are hazardous, because CO_2 replaces oxygen. Too much CO_2 also has a negative impact on human comfort.

Normally, the amount of released CO_2 during fermentation is a direct indicator of fermentation activity of yeast. The CO_2 content of beer is one of its most important quality criteria. Beers with a good amount of foam have a CO_2 content of 0.45 - 0.50% about 15% of the CO_2 content produced remains dissolved in beer. It is particularly important to measure CO_2 safety levels in *tella*. Because CO_2 is heavier than air the gas can be accumulate in non-ventilated area and pose a serious health risk for consumers. It influences taste, acidity, density, and pH values of *tella* [4, 27, 28]. According to EBC and the range of CO_2 in beverages must < 0.5%.

Table-A: Different analytical *tella* parameters with their analytical techniques and guideline values of EBC standard.

Tella parameters	Techniques used	Potential health effect	EBC
Alcohol contents	Alcoholmeters	Health risk	2-8%
pH	pH meter	Affects mucous membrane; bitter taste; corrosion	4 - 5
Titratable acidity	titration	Cause dental erosion	0.16 - 0.8%
Carbondioxid	titration	Affect pH of body, taste, color of *tella* and health risk	<0.5%
Microbial loads	serial dilution and spread plate	Cause spoilages, change flavor, pH, liver damage	30-300 CFU/mL

3. Materials and Methods

3.1 Description of the Study area

Study was conducted in Jimma city, which is the capital of Jimma Zone, Oromia regional state, Ethiopa. It is located at 355 km from the Addis Ababa, in Southwest Ethiopia. It is available at latitude and longitude of $7^{o}40'N36^{o}50'E$ and altitude, of about 173 m above sea level. It lies in the climate zone locally known as "weyna dega" which is ideal for agriculture as well as human settlement.

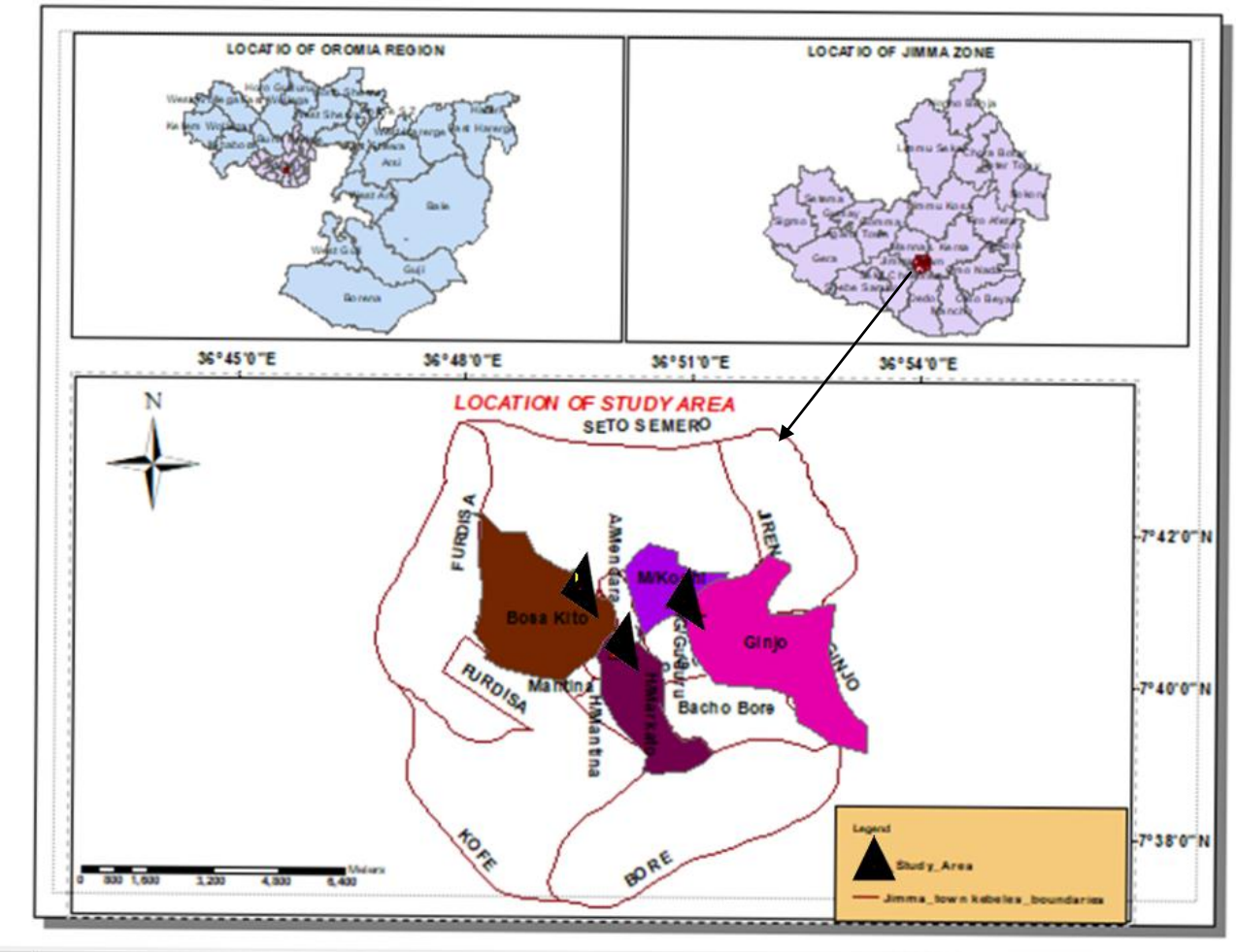

Figure-2: Shows map of the study Jimma zone and City

3.2 Sampling area and sample collection

Study was conducted in Jimma city, which is the capital of Jimma Zone, Oromia regional state, Ethiopa. Sample areas were selected due to considerations of widely fermentations and consumptions areas of *tella*. Thus, purposive sampling was considered to select three localities from Jimma city namely *Matric Sefer, Kochi* and *Merkato tella*. From sampling area, totally 19 *tella* samples, were collected in the morning using sterilized polyethylene bottles from different *tella* vending houses. Each day, *tella* samples were collected for analysis of the physicochemical parameters and microbial load. All the collected samples were kept in fridge at 4 °C for each until microbiological and physicochemical analysis was carried out in the laboratory.

3.3 Chemicals and apparatus

Chemicals: Sodium hydroxide pellets (Steinheim, Germany), peptone and salanin(cod 64271 Germany), phenolphthalein indicator from UNICHEM Chemical reagent , buffers of pH 4 and 7 (Merck), Violet Red Bile Glucose Agar (VRGA), Plate Count Agar (PCA), de Man, Rogosa and Sharpe Agar (MRS) (Madrid, Spain), Yeast extract Glucose Chloramphenicol (YGC)(Spain) were used during the experiments.

Instrument: Portable pH meter code 013(Germany), Alcoholmeter Gay Lussac, Abbe refractometry cod 330 (Japan), Colony forming counter (Funke Gerber Code 2013, Switzerland) were used during the experiments.

3.4 Preparation of standard

0.4g of sodium hydroxide would be dissolved in 100 mL of distilled water to make 0.1M of NaOH would be performed.

3.5 Determination of pH

The pH of *tella* samples was determined in the laboratory using a portable digital pH-meter after pH meter calibrated. The pH meter instrument (glass) and reference electrode was calibrated by using known standard buffer solution of pH 7.0 and 4.0. The electrode was removed from the

buffer solution and rinsed with distilled water and dried by gently blotting with a soft tissue paper. Finally, the electrode was immersed in the beaker containing *tella* and the pH reading was done directly from pH- meter. The pH-meter was calibrated before and after the samples were measured [23].

3.6 Determination of titratable acidity (TA)

Titratable acidity was determined using AOAC method [25, 28]. Accordingly, 10 mL *tella* sample was pipetted into a beaker and then, 3 - 5 drops of 1% phenolphthalein indicator was added. *Tella* sample was then titrated with 0.1 M NaOH solution, until a faint pink color was appeared. Finally, titratable acidity of *tella* samples, which was expressed as percentage of conversion factors like acetic acid 0.06, citric acid 0.064, tartaric acid 0.075, and lactic acid 0.09 was calculated as the as % of acetic acids in *tella* by the following formula [28].

$$\% \text{ TA} = V_{NaOH} \text{ x conversion factor.}$$

Where V- volume of NaOH consumed, conversion factor acetic acid 0.06

3.7 Determination of carbondioxid content

A carbon dioxide content of *tella* was determined by EBC method [27]. Accordingly, 10 mL of the *tella* was pipetted into a beaker and calibrated pH immersed in beaker. Then, 3 - 5 drops of 1% phenolphthalein indicator was added. *Tella* sample was then titrated with 0.1 M NaOH solution up to pH 8.3 till the pink colors were persist indicating the end point. By recording the amount of sodium hydroxide consumed to reach the end point the amount of carbon-di-oxide in *tella* was calculated by [29, 30]. Calculation: $CO_2 (\%) = \frac{V_t \text{ x } M \text{ x } 44}{V_s \text{ x } 100}$

Where, V_t - volume of NaOH consumed, M= molarities of NaOH, V_s - Volume of *tella* taken.

3.8 Determination of alcohol contents

The alcohol content of *tella* was measured by using an alcoholmeter in the laboratory after calibrated by ABV 96-99% of ethanol, ABV 5% of Beddelle beer and ABV 4.5% of Dashin beer [31].

3.9 Determination of the refractive index

All the refractive indices have measured by using a refractometry on to which a water bath was attached. The function of the water bath is to keep the temperature of the system constant (at 20 ºC). Before and after each measurement of the refractive index of each sample, the refract meter has calibrated with distilled water [6, 32].

3.10 Microbial load analyzing

3.10.1 Procedures for preparation of materials used to collect tella samples

First material by which *tella* collecting was sterilized at121°C for 15 min. This used to kill any microorganism from materials. *Tella* was collected by sterilized polyethylene bottles.

3.10.2 Procedures for preparation of media and plates

The nutrient agar and peptone were used for preparation. The procedure was as follows; 28 g of nutrient media powder was weighed out and dispersed in 1 L of distilled water and allowed to soak for 10 mins in a conical flask. The mixture was swirled to mix, and then sterilized for 15 mins at 121 ^{0}C. The mixture was cooled and mixed well [4]. It was poured into Petri-dishes and allowed to cool down to 40-42 °C.

3.10.3 Procedures for platting media and samples on different plates

From collected *tella*, 10 mL of *tella* was taken and mixed with 90 mL peptone prepared, then homogenized for 35 second speedily. From mixture 1 mL was taken then mixed with 10 mL of salanin in test tube by micro pipette. From test tube 0.1 mL was taken and serially diluted on 9.9 mL of salanin by sterilized discrete pipette. The serial dilution was done in the range of 10^{-1} to 10^{-8} series of test tubes.

For the estimation of total Anaerobic Mesophilic Bacteria (AMB), the sample of 1 mL was taken from the test tube aseptically using discrete pipette and plated on Plate Counting Agar (PCA). Then, plates were inverted. Finally, all plates were incubated in oxoid jar at 32 °C for 48 hrs. For the estimation of total (EB) population, the sample of 1 mL was aseptically taken from the test

tube using sterilized discrete pipette and was placed on duplicate Violet Red Bile Glucose Agar (VRBGA) plate. Finally, the plates were placed inverted and incubated at 32 °C for 24 hrs. For the estimation of yeast, the sample of 1 mL was aseptically taken from the test tube using sterilized discrete pipette and was placed on duplicate Yeast extract glucose Chloramphenicol (YGC) plate. Then, inverted and incubated at 27 °C for 3 to 5 days. For Lactic Acid Bacteria (LAB), volumes of 1 mL of the appropriate dilutions were spread-plated on pre-dried surfaces of duplicated on de Man, Rogosa and Sharpe Agar (MRS) plate, and incubated under anaerobic conditions using anaerobic jar (oxoid jar) at 30 °C for 48 to 72 hr. Grouted colonies were counted using digital colony counter. At the end microbial loads of *tella* were calculated by [32].

CFU = number of colony / dilution factor x volume of sample used and given by CFU/mL unit.

3.11 Data processing

The results were presented using the Analysis of Variance [ANOVA software version 20 and Microsoft Office Excel, 2007]. There were triplicate measurement for each physicochemical parameters and microbial loads determinations. Means and standard deviation were compared by ANOVA and tested by least significant difference (Lsd) at 5% level. Descriptive statistics [means and standard deviations] were used to summarize the data on microbial numbers, CO_2, alcohols, pH, titratable acidity and refractive index of tella scores. Data on microbial counts were first transformed to logarithmic [$\log_{10}$] values before computing the mean counts and standard deviations. Furthermore, significant differences among the mean values were determined by using the analysis of variance (ANOVA) and range test was conducted at a significance level of $p < 0.05$.

4 Results and Discussion

4.1 Physicochemical properties of *tella*

The results of phyisco-chemical properties of the *tella* from different areas have been summarized in Table-1. Based on the results, discussions were made accordingly for each physicochemical parameter. For each sample, the measurements were done in triplicate and the results were noted as mean ± SD.

Table-1: Alcoholic content and other parameters of Jimma city *tella*

	***Tella* sources**				
Parameters	***Kochi***	***Matrik sefer***	***Merkato***	**EBC [27]**	**Beddelle Beer factory**
Alcohol	2.72 ± 0.09	2.01 ± 0.24	2.42 ± 0.10	2-8	≤5%
pH	3.92 ± 0.05	4.46 ± 0.02	4.1 ± 0.10	4-5	4-4.5
%CO_2	0.32 ± 0.06	0.19 ± 0.03	0.21 ± 0.04	< 0.5	< 0.5
%TA	0.202 ± 0.01	0.144 ± 0.02	0.19 ± 0.01	0.16-0.8	0.1-0.6
Refractive index	1.338 ± 0.01	1.342 ± 0.01	1.339 ± 0.01	-	-

EBC=European Beer Convection, TA=Titrable Acid (% acetic acid). Values are means of triplicate determinations.

4.1.1 Alcohol contents

Table 1 show that the alcohol content of *tella* samples collected from *Kochi* was significantly higher ($p < 0.05$) than those of *Merkato* and *Matrik sefer tella*. In this study, the alcohol content of some *Matrik sefer tella* was not within the normal EBC range recommended. This might be due to CO_2 produced from the yeast cannot escape *tella* as it did during the first fermentation, so *tella* becomes carbonated, and small amounts of alcohol may be produced or ingredients not fermented well [33, 34].

Significantly higher in alcohol content of *Kochi tella* than *Matrik sefer tella,* was accompanied by yeast growth and decrease in reducing sugars and total carbohydrates. But, high levels of alcohol drop pH that make *tella* a poor substrate like sugar and total carbohydrates for most microorganisms especially yeasts. This is because the alcohol stress can reduce yeast cell volume [34, 35]. Significantly high carbohydrate content coupled with the small amount of alcohol contents of *tella* serve as good source of energy [13]. Moreover, conditions such as temperature, time of fermentation, types of ingredients used, procedures of *tella* preparation and handling as well as strains of the microorganisms obviously affect the alcoholic level of *tella* [10, 36].

4.1.2 pH

Table 1 shows that the pH values of *tella* samples collected from *Kochi* was significantly lower ($p < 0.05$) than those of *Merkato* and *Matrik sefer tella*. The mean average pH of *tella* samples obtained from *Kochi*, *Matrik sefer* and *Merkato*: 3.92 ± 0.05, 4.46 ± 0.2 and 4.1 ± 0.1, respectively, were pH of *Kochi tella* not within the normal pH range of good *tella* and EBC recommended. This indicated that there were bacterial growths in *tella* samples as well as due to the consumption of ammonium ions, potassium ions and amino acids by yeast and the consequent release of hydrogen ions and secretion of organic acids [34]. The organic acids, including acetic and lactic acid produced by the actions of the yeast and lactic acid bacteria. Significantly less pH value causes *Kochi tella* to decrease of general sensory quality and unacceptable ranges, which was similar to the reports of [37], where he observed that much lactic acid was produced during the fermentation of masa which led to a progressive fall in pH and gives the product a sour taste. However, the mean average pH of *tella* samples obtained from *Matrik sefer,* 4.46 ± 0.2 and *Merkato,* 4.1 ± 0.1 was within normal range of pH of fresh *tella* indicating that *tella* samples significantly less microbial loaded.

4.1.3 Titratable acidity (TA)

There is significantly higher value in titratable acidity of *Kochi* and *Merkato* than *Matrik sefer tella* (Table-1). This is might be due to EB which would normally initiate fermentation was

suppressed by rapid decrease in pH with accelerated increase in acidity followed by high growth rate of LAB responsible for end fermentation.

Table 1 show that there is significantly lower in titratable acidity 0.144 ± 0.02 and significantly higher pH 4.46 ± 0.2 values of *Matrik Sefer tella*. This is might be come from the precipitation of phosphates and amino acids/polypeptides derived from the malt during mashing and the destruction of some microbial cells like EB probably to competition amongst the LAB strains and nutrient depletion of the fermenting samples hence reducing the quantity of acid produced [9, 38]. In present study, the percentage TA in *Kochi*, *Matrik sefer* and *Merkato tella* samples, was found in EBC and Beddelle beer factory guide line. In general, the titratable acidity (TA) values were significantly influenced by the days of storage both at refrigeration and room temperature. As well as significantly difference in traditional methods of *tella* production which are none standardized in terms of raw materials, equipments, time of fermentation, finished products quality and handling brings the difference in acidity of *tella* [2, 25, 37].

4.1.4 Carbondioxid contents

Table 1, show that there was significantly difference ($p < 0.05$) in CO_2 contents among sampling sites. There is significantly higher content of CO_2 0.32 ± 0.006, in *Kochi tella* than *Markato* and *Matrik sefer tella*. This due to carbonation which occurred by smoking materials to achieve desire level of product may increase the contents of CO_2 in *tella* beverages. Also, since CO_2 dissolution in beverages principally depends on temperature, high amount of CO_2 cannot dissolve in *Kochi tella* [27]. From the study, significantly high amount of CO_2 in *tella* indicate the activity of yeasts. But, significantly higher concentration of CO_2 affects the growth and multiplication of yeast due to the formation of carbonic acid (HCO_3^- ions) that reduce the pH of the yeast medium, that make significantly low loads of yeast in *Kochi* than *Merkato tella* [28, 39]. In this study, contents of CO_2 in collected *tella* were in range of EBC and Beddelle beer recommended.

4.1.5 Refractive indexes

Refractive index of collected *tella* has significant difference ($p \leq 0.05$) among *tella* sample site (Table-1). *Matrik sefer tella* has significantly higher refractive index (1.342 ± 0.001) than *Kochi* and *Merkato*. This is could be due to the difference in fermentation time of *tella*. *Tella* that contain significantly large refractive index have large number of suspended solids, unfermented sugar, form small alcohol and titratable acidity (TA). In general, storage of the beverages under ambient temperatures for various lengths of time resulted in increase titratable acidity and alcohol content and decrease in the pH values, CO_2 contents and refractivity.

4.1.6 Microbiological analysis of Jimma city *tella*

The results of microbiological analysis of the *tella* from different areas have been summarized in Table-2. Based on the results, discussions were made. For each sample, the measurements were done in triplicate and the results were noted as mean ± SD.

Table-2: Microbial loads of *Kochi*, *Merkato* and *Matrik Sefer tella* in log CFU/mL

	***Tella* sources**			
Microbials	***Kochi***	***Matrik sefer***	***Merkato***	**EBC standard [31]**
LAB	8.01 ± 0.14	6.65 ± 0.03	7.24 ± 0.04	$< 10^9$
EB	1.1 ± 0.01	2.02 ± 0.01	1.14 ± 0.01	$< 10^3$
AMB	4.28 ± 0.03	5.5 ± 0.04	4.64 ± 0.03	$< 10^7$
Yeast	6.28 ± 0.04	5.09 ± 0.01	6.74 ± 0.04	$< 10^7$

LAB=Lactic Acid Bacteria, EB=Enter Bacteria, AMB=Anaerobic Mesophilic Bacteria.

The result obtained showed that, there was significant difference ($p < 0.05$), in microbial loads among house of *tella* venders and sample site. *Kochi tella* has significantly higher LAB, than *Merkato* and *Matrik sefer tella*. This is indicating fermentation time *tella* and malt contained a

considerable number of LAB and yeasts which are considered as the primary loads' microbes in carbonated products mainly due to their ability to tolerate low pH and acidic conditions [40, 41].

Table 2 shows that there was significance difference ($p < 0.05$), in EB count among *tella* samples from *Merkato*, *Kochi* and *Matrik sefer*. In the current study, significantly higher EB counts obtained from *Matrik sefer tella* 2.02 ± 0.002, than other sites. This indicates the contamination of *tella* samples either from the poor hygiene or improper handling of *tella*. This indicates poor microbial quality of *tella* [37, 42]. *Kochi tella* has significantly lower EB 1.1 ± 0.001, than another site. This is due to high alcohol contents and acidic nature of *tella* was not favorable for its rapid multiplication and its acidic intolerant microbial [43]. The result also revealed that, there is significantly a smaller number of AMB from *Kochi* and *Merkato tella*, this is due to high LAB present inhibits AMB spore forming bacteria [37]. Yeasts from *Kochi tella* was significantly less than that of *Markato tella,* since, high alcohol stress can reduce cell volume of yeast and death of yeast release CO_2 that favorable for LAB and lower pH [4, 34].

Significantly low microbial loads from *Matrik Sefer tella* was, due to over darkening of the roast may lead to loss of the sugar content of the ingredients and deep roasting of ingredients which used for coloring *tella* as well as the presence of large amount of *gesho* inactivates the contaminants [18]. In addition to this the entire unmalted ingredients used in production of *tella* were cooked at various phases by roasting, steaming or boiling at different temperatures and holding times. These temperatures would be expected to eliminate microbial cells, except the thermo-tolerant LAB [21, 44]. Low availability of oxygen inside the tank; may be oxidized ethanol to acetic acid, due to the growth of Acetobacter, which convert ethanol to acetic acid (Ethanoic acids) under aerobic condition.

$$CH_3CH_2OH + 2[O] \rightarrow CH_3COH + 2[O] \rightarrow CH_3COOH + H_2O$$

Ethanol Oxygen acetaldehyde Ethanoic acids (acetic acids)

Acetic acid is the most abundant of the volatile acidic constituents of *tella*. Acetic acids are acid-tolerant bacteria. Most are growing at pH 3.6–3.8, and some even at pH 3. Also, yeast is known to produce minor amounts of acetic acid in fermentation under anaerobic conditions which spoiling *tella* [45].

5. Conclusion

The result revealed that, there were significant differences in physicochemical parameters and microbial load of *tella* in the study areas. The result indicated that lactic acid bacteria and yeasts were involved largely in the fermented *tella*. The study indicated that all the physicochemical and microbial loads of *tella* were within EBC and Beddelle beer factory permissible limit, except pH values of some *Kochi* and *Merkato tella* below recommended values. Based on the results, the differences among households in combination of malt and unmalted ingredients, source of water (river, well, rain fall, tap), hygienic practices, fermentation time and utensil used may result in variations of microbial count and physicochemical properties of *tella.* The pH and alcohol content were in the range of 4 - 5 and 2 - 8% (v/v) respectively, when *tella* is considered to be the most suitable for consumption. In the present study the microbial load trends were, LAB > yeasts > AMB > EB. All collected *tella* samples have pH values ≤ 4.5; this indicates *tella* is slightly acidic. The alcohol contents of collected *tella* samples were ranged from 1.77-2.81%v/v and titratable acidity (TA) ranged in 0.124-0.203% acetic acids. Therefore, it is advisable that *tella* when produced should be consumed freshly.

5.1 Recommendation

Depending on the study the researcher recommended the following points.

- Future work shall focus on safety, raw material types to commercialize *tella* and market research of *tella*.
- Future work shall focus on types of ingredients used to make *tella* with their ratio, nutritional values and anti-oxidant capacity of *tella* that lead higher acceptance of *tella* as diet beverages.
- Another, further studies are necessary on the isolation and role of different types of microorganisms at different stages of *tella* fermentation and at different pH values for making good acceptance and quality of *tella.*
- This research was done on *tella* beverages by collecting them on the matured date/ready to drink. So anyone who has interest can do investigations, the effect of under maturations and long shelf life on *tella* bioactive chemicals.

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APPENDEXY: A





Fig-2: sample in freezes **Fig-3:** TA and CO2 by titration **Fig-4:** pH determination **Fig-5:** Alcoholmeter **Fig-6:** Refract meter






Fig-7: Samples for microbial load testing **Fig-8:** PCA, YGC and VRBGA **Fig-9:** Incubation in anaerobic jar





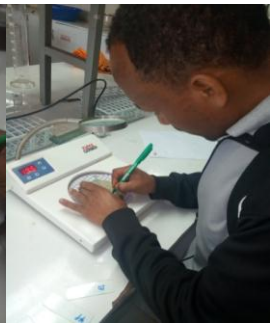

Fig-10: Grouted Microorganisms on different Medias **Fig-11:** Counting organisms by colony counter.

APPENDEX: B

ANOVA

Alcohol

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.949	2	.474	15.466	.000
Within Groups	.368	12	.031		
Total	1.317	14			

ANOVA

Ph

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.833	2	.417	4.879	.028
Within Groups	1.024	12	.085		
Total	1.857	14			

TA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.019	2	.010	85.818	.000
Within Groups	.001	12	.000		
Total	.020	14			

CO_2

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.037	2	.018	88.458	.000
Within Groups	.002	12	.000		
Total	.039	14			

Refra.index

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.000	2	.000	8.622	.005
Within Groups	.000	12	.000		
Total	.000	14			

LAB

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	20.463	2	10.231	181.127	.000
Within Groups	.678	12	.056		
Total	21.140	14			

ANOVA

Yeast

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	4.434	2	2.217	51.082	.000
Within Groups	.521	12	.043		
Total	4.954	14			

AMB

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	9.011	2	4.506	733.801	.000
Within Groups	.074	12	.006		
Total	9.085	14			