

**STUDIES ON INDIGENOUS PREPARATION PRACTICES, EFFECTS
OF INGREDIENTS, FERMENTATION TIME AND PASTEURIZATION
ON QUALITY AND SHELF STABILITY OF *TELLA***

MSc Thesis

By

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June, 2016

Jimma, Ethiopia

**STUDIES ON INDIGENOUS PREPARATION PRACTICES, EFFECTS
OF INGREDIENTS, FERMENTATION TIME AND PASTEURIZATION
ON QUALITY AND SHELF STABILITY OF *TELLA***

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*In Partial Fulfillment of the Requirements for the Degree of Master of
Science in Postharvest Management (Perishable)*

Major advisor- Yetenayet Bekele (PhD)

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Jimma, Ethiopia

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DEDICATION

This thesis manuscript is dedicated to my dearly loved mother Shitaye and wonderful brother Abayneh.

STATEMENT OF THE AUTHOR

I, the undersigned, declare that this Thesis is my work and is not submitted to any institution elsewhere for the award of any academic degree, diploma or certificate and all sources of materials used for this Thesis have been duly acknowledged. This Thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Jimma University, College of Agriculture and Veterinary Medicine and deposited at the University Library to be made available to borrowers under the rules of the library.

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BIOGRAPHICAL SKETCH

The author Shilimat Tolossa was born to her mother W/o Shitaye Dadi and her father Ato Tolossa Dube on September 1989 G.C in *Tolay, Southern Ethiopia*. She attended her Elementary and junior high school education in Addis Ababa at *Yemane Birhan* School from 1995-2002 GC and her high school education at *Ayer Tena* School from 2003-2004 GC. Upon completing her high school education, she attended her preparatory education at *Kefitegna 4* School from 2005-2006 G.C. Shilimat Tolossa started her university education at Mekelle University in October, 2007 G.C. and graduated with Bachelor of Science degree (BSc) in Horticulture in June 2010 G.C. From August, 2011- October, 2012 she was employed by Ethiopian Horticulture Development Agency as a junior horticulture expert. She joined Jimma University College of Agriculture and Veterinary Medicine in October, 2012 G.C to pursue her post graduate study in Postharvest Management.

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Finally, I would like to extend my heartfelt gratitude to my husband Logan Hamby for the love and encouragement. Thank you for being patient with me.

LIST OF ABBREVIATIONS

AOAC	Association of Analytical Chemists
ANOVA	Analysis of variance
BPEDROS	Bureau of planning and Economics Development of Oromia Regional State
°C	Degree centigrade
CFU	Colony forming unit
CRD	Complete randomized design
g	Gram
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
Kg	kilogram
NaOH	Sodium hydroxide
m.a.s.l	Meters above sea level
min	Minute
ml	Milliliter
MRS agar	de Man, Rogosa and Sharpe agar
PU	Pasteurization unit
TA	Titrateable acidity
TSS	Total soluble solids
W/V	Weight by volume
SPSS	Statistical Package for Social Science
SAS	Statistical Analysis Software

GLOSSARY

<i>Asharo</i>	An adjunct prepared from flour of roasted barley
<i>Atella</i>	Spent of <i>tella</i>
<i>Bikil</i>	Malt
<i>Biret mitad</i>	Metal griddle pan used for baking
Derekote	An adjunct prepared from flour of roasted maize
<i>Difdif</i>	Thick slurry formed on the second phase of fermentation which is a mixture of water, local hops, malt and additional substrates
<i>Enkuro</i>	Additional substrate used for the preparation of <i>tella</i> which is prepared from flour of maize or barley mixed with water and toasted on a metal griddle
<i>Gesho</i>	Local hops (<i>Rhamnus prinoides</i>)
<i>Grawa</i>	Bitter leaves (<i>Vernonia amygdalina</i>)
<i>Kebele</i>	The lowest administrative structure in Ethiopia
Kirari	Drink made from spent of <i>tella</i> by adding water and allowing it to ferment for one or two days
<i>Sefed</i>	Traditionally woven round tray
<i>Tella</i>	An Ethiopian indigenous fermented alcoholic beverage
<i>Tinsis</i>	Mixture of pounded <i>gesho</i> leaves and water used as a starter for <i>tella</i> making
<i>Weira</i>	Amharic term for <i>Olea- europaea subsp. Cuspidate</i>
<i>Yedingay wefcho</i>	Traditional grinding tool made from stone
<i>Yetella kita</i>	Flat bread prepared from flour of wheat/maize/barley/millet or a combination of them

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ABSTRACT

*Tella is one of the Ethiopian traditional fermented beverages prepared from different malted and unmalted grains. It is brewed from local hops (*Rhamnus prinoid*), malts of barley, maize, wheat, sorghum, finger millet and even from teff in some parts of the country. Tella being the most consumed indigenous beverage in Ethiopia it's the least investigated in terms of effects of ingredients and shelf stability. Therefore it is important to study effect of different ingredient compositions on physicochemical properties and pasteurization on the shelf stability of tella in order to improve its keeping quality. This study aimed at assessing the traditional processing of tella and improving its shelf life stability. The study was carried out in three phases. During phase one, a survey was conducted in five purposively selected kebele's of Jimma town: Bosa addis, Bosa kito, Merkato menahariya, Merkato mentina and Becho bore to assess the indigenous knowledge regarding its processing. A semi structured questionnaire was used to gather relevant information and SPSS (version 20) was used to analyze the survey data. Phase two evaluated the effect of malt combination, adjunct combination and fermentation duration on physicochemical properties of tella. It was a three factorial having each factors with three levels in three replication laid out in RCBD design. During phase three, a time-temperature study was carried out and two pasteurization temperatures, 15.86 PU at 60°C and 7.79 PU at 65°C were selected. The effect of pasteurization on the physicochemical properties, microbial and sensory qualities of tella were studied. SAS (version 9.2) was used to analyze data from phase 2 and 3. The survey result showed that 100% of the respondents were women who were in the age group of 31-40 (30%) and the majority (51.5%) had been in tella selling business for more than 10 years. Physicochemical properties of tella were significantly ($P<0.05$) affected by malt, adjunct combination and fermentation time. The changes in pH, TSS, TA, alcohol content of pasteurized tella samples were much slower during the storage period. Result of the sensory evaluation showed that there was no significant difference between unpasteurized and pasteurized samples at 60°C in terms flavor, aroma, mouth feel and overall acceptability. Samples pasteurized at 65°C scored the lowest mean for flavor, taste, color, mouth feel and over all acceptability. Pasteurization had a significant effect on microbial load ($P<0.05$). Unpasteurized samples had the highest microbial growth during the storage period. While pasteurized at 60°C and 65°C had no microbial growth on day one and maintained a very low microbial growth during the storage period. The microbial load of pasteurized sample was initially zero which later showed a small change during storage. Control samples had higher microbial count compared to pasteurized samples. Pasteurization extended shelf stability of tella for 45 days without a significant change in terms of physicochemical and microbial changes. Effect of pasteurization at higher temperature needs further studies.*

Key words: Tella, fermentation, pasteurization, shelf stability, quality, traditional beverage, Physicochemical properties, microbial content, adjuncts,

1. INTRODUCTION

1.1. Background

While no one knows when alcoholic beverages were first made and used, it was most likely the result of accidental chance that occurred at least tens of thousands of years ago. However, the discovery of late Stone Age beer jugs has established the fact that intentionally fermented beverages existed at least as early as the Neolithic period (10,000 B.C.). In ancient times people always drank when holding a memorial ceremony, offering sacrifices to gods or their ancestors, pledging resolution before going into battle, celebrating victory, before feuding and official executions, for taking an oath of allegiance, while attending the ceremonies of birth, marriage, reunions, departures, death, and festival banquets (Dwight, 1995).

Productions of traditional beverages provide a multitude of individuals and households with livelihood, either as the sole means of their sustenance or a source of critical supplemental income. Through the years, most developed countries have managed to bring the production of alcoholic beverage under state supervision, thus standardizing the production process, inputs, as well as the concentration of alcohol. However, in many developing countries, particularly in Africa, they are produced traditionally at the homestead provide the bulk of the alcohol consumed at the local level (WHO, 2004).

In East Africa, the consumption of indigenous alcoholic beverages accounts for 80% of the entire consumption. In some of those areas, alcoholic beverages constitute payment for labor and serve as an important cash income for women (Kubo, 2014).

The production and consumption of alcoholic beverages has a long history in Ethiopia. In fact some studies state that the highland areas of Ethiopia were among the first seven centers in the world where plants were grown for alcohol production (Yeraswork and Ezana, 2010).

In Ethiopia, villagers prepare a wide range of fermented beverages from different raw materials. Some of the known Ethiopian traditional fermented beverages are *tella*, *tej*, *borde*, *shamita*, *korefe*, *kineto* and *bukire* (Kebede *et al.*, 2002).

Tella is one of the Ethiopian traditional alcoholic beverages, which is prepared from different ingredients. It is brewed from various grains and different cereals which include; barley, corn,

wheat and sorghum and also from teff although in some regions, millet and *Rhamnus prinioids* (Fite *et al.*, 1991).

1.2. Statement of the Problem

Very few reviews and research works have been done to study and document *tella processing*. Its preparation and physicochemical properties were studied by few researchers (Fite *et al.*, 1991; Gizaw, 2006; Fekadu, 2013; Lee *et al.*, 2015). The microbial profile was also studied (Samuel and Berhanu 1991; Berhanu, 2014). Belay and Awraris (2014) tried to modify the fermentation process to improve physicochemical and sensory quality. No works has been done to extend the shelf life of *tella*. The shelf-life of *tella* is very limited and results economic loss to brewers who depend on selling *tella* as a means of income generation. Therefore, it is important to carry out studies which will contribute to the improvement of the beverage in terms of sensory and microbial quality, safety and shelf life stability.

The present study was intended to document the indigenous processing methods and study the effect of pasteurization on the physicochemical properties, sensory qualities and shelf stability of *tella*. The study contributes essential findings to reduce loss and extend its shelf stability which will help small scale brewers to scale up their production. The findings will also serve as significant input for the commercializing of the beverage.

1.3. Research questions

1. How is *tella* traditionally processed?
2. How does the composition of ingredients affect the physicochemical properties of *tella*?
3. How does pasteurization extend the shelf life of *tella*?
4. What is the effect of pasteurization on the physicochemical properties, sensory quality and microbial profile of *tella*?

1.4. Objective of the study

1.4.1. General objective

To assess indigenous processing practices, effect of ingredient composition, fermentation time and pasteurization schedule on quality and shelf stability of *tella*

1.4.2. Specific objectives

- I. To document the existing traditional preparation method of *tella*
- II. To study effects of ingredients composition on physicochemical properties of *tella* under local preparation conditions
- III. To develop pasteurization time-temperature
- IV. To evaluate the effect of pasteurization on physicochemical properties, microbial content, sensory qualities and shelf stability of *tella*

2. LITERATURE REVIEW

2.1. *Tella*

Tella is one of the Ethiopian traditional alcoholic beverages, which is prepared from different ingredients. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia. It is estimated that over two million hectoliters of *tella* to be brewed annually in households and drinking houses in Addis Ababa alone. The detail of *tella preparation* differs among the ethnic groups and depends on traditional and the economic situation. The clay container (*insera*) is washed with *Grawa* (*Vernonia amygdalina*) and water several times and after that smoked with wood from *Weira* (*Olea europaea subsp. Cuspidate*) for about ten minutes. Germinated grains (malt) of barley, or corn, or wheat (*bikil*), bought in the local market or prepared at home, are dried and milled. For making *bikil*, the grains are moistened in water and the moist grains are placed between fresh leaves of false banana (*Enset ventricosum*), left to germinate for 3 days and after that dried. Dried *gesho* (*Rhamnus prinoids*) is available in the local market. The leaves of *gesho* are dried again in the sun for about ½ hour and after that pounded. The leaves are separated from the stems, which need a longer time to dry. The pounded *gesho* leaves are placed in a clay container with water and left to ferment for 2-3 days. *Gesho* is responsible for the bitter taste of *tella* and the bitterness of *tella* is directly related to the amount of *gesho* added during brewing. It is also thought to be the source of various chemicals to improve flavor of *tella* (Sahle and Gashe, 1991).

Some of the grains intended for *tella* preparation are roasted and milled, and then mixed with water and baked on the *mitad* to prepare what is known as *kita* (a thin, 5-10 mm thick, pancake-like bread). This *kita*, broken into small pieces, part of the milled *bikil* and the pounded *gesho* stems are added to the water and allowed to ferment for 1-2 days. The rest of the flour is toasted on *mitad*, sprinkled with water and toasted until dark brown to form what is known as *enkuro*. The mixture *enkuro*, malt (*bikil*), some *gesho*, and water are added to the container. The mixture is kept covered overnight, after which more water is added and the container is kept sealed for 5-7 days, until the beverage is ready. High quality *tella* is made with a relatively small quantity of water (Gizaw, 2006).

There are several recipes for making *tella* and it appears as if every housewife has her own version of the recipe. The fermenting microorganisms of *tella* are composed of *Saccharomyces cerevisiae* and *Lactobacillus* species. The pH of *tella* is in the range of 4.5 to 4.8. 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 (Samuel and Berhanu, 1991). According to their study, the fermentation process of *tella* is divided into four phases. The first occurs in the original mixtures of ingredients and the second and third phases occur after successive additions of more carbohydrate materials. The three main carbohydrate materials are mentioned to be *bikil*, *kitta* and *enkuro*. The latter phase is where acidification takes place, which is actually not desirable. Maximum ethanol production occurs during the third phase and at the beginning of the fourth phase. Filter *tella* is another beverage, which is made in the same way as the regular *tella*, but it is more concentrated and filtered through a cotton cloth. After being prepared it is kept in a closed container. *Kirari* (which is of less quality) is a drink made from a left over crude after clear *tella* is used, by adding fresh water and then leaving the mixture to ferment. This beverage is weaker than the regular *tella*, and is most often used for family consumption.

2.2. Main Ingredients of *Tella*

2.2.1. Gesho (*Rhamnus prinoides*)

Rhamnus prinoides (Amharic, *Gesho*) is in the family *Rhamanceae*. It is a wide spread plant species in east and south African countries. The only two *Rhamnus* species that occur in Africa are *R. prinoides* and *R. staddo*. *Rhamnus prinoides* is common in many parts of eastern and central Africa. The plant is native to Ethiopia, Botswana, Eritrea, Lesotho, Namibia, South Africa, Swaziland, Uganda and it is exotic to Kenya. It also occurs in Cameroon, Sudan, and Angola (Afewerk and Bhagwan, 2012).

Gesho is cultivated in Ethiopia in view of its important application in the domestically brewed beverages. *Gesho* is an important commodity which is sold in almost every traditional market in Ethiopia. Although it is quite common to find *Gesho* cultivation throughout the country, it is worth mentioning that in Tigray region, around *Kara Kori* in North *Shoa* and *Sebeta*, just west of Addis Ababa, are important centers of production. Berhanu and Teshome (1995)

investigated the chemical constituent of gesho leaves. The result showed that β -sorigenine-8-O- β -D-glucoside, a naphthalene compound is responsible for the bitter taste of the leaves. *Gesho* is used to impart the characteristics bitter flavor to domestically brewed beverages, *tella* and *tej*. It also regulates the micro flora responsible for the fermentation process. It plays a major role to suppress certain spoilage/pathogenic bacteria during the fermentation process (Ararso and Alemayehhu, 2013).

2.2.2. Malt (Bikil)

Tella brewing process like brewing of beer requires malt as a main ingredient. Malt can be prepared from different cereals such as barley, wheat, millet or mixture of those. In the brewing process, the principal enzymes responsible for starch conversion are amylases. They can degrade starch into maltose, glucose and other forms of carbohydrates. They convert the available starch to fermentable sugars. The source of these enzymes in many types of beer production is clearly known (it originates from malt) while in the process of production of *tella* the enzyme source is not yet investigated and well documented but is believed that it comes from malts (Berhanu, 2014).

2.2.3. Adjuncts used in *tella*

Enkuro is made from dry roasted corn or barley, grounded into fine flour and sprinkled with water and subsequently toasted using big flat metal pan. *Kita* is a flatbread prepared from flour of grains like corn, wheat, barley separately or in mixture. *Derekote* is another substrate which is prepared from grains such as corn, wheat, or barley that has been roasted on pan until the color changes into brown. These substrates serve as a source of carbohydrate in the fermentation process and for dark or dark brown color (Berhanu and Amare, 2013).

2.3. Cleansing Agents Used During *Tella* Preparation

2.3.1. Grawa (*Vernonia amygdalina* Del)

Grawa is popularly known as bitter leaf. It belongs to the *Compositae* family. It is widely distributed in most African countries and utilized for various purposes (Okafor, 1983). In some parts of Africa it is regarded as a medicinal plant to treat fever, kidney problems and stomach discomfort (Hamowia and Saffaf, 1994). Additional studies showed that it has bactericidal effects and antifungal properties (Erasto *et al.*, 2006; Iwalokun *et al.*, 2006).

In Ethiopia *Grawa* is used as ethno medicinal plant for stomach disorders and as a cleansing agent for containers of *tella* and *tej* fermentation. Containers are washed repeatedly using *Grawa* leaves and water till foams appear. The effect of *Grawa* leaves in relation to *tella* and *tej* preparation is not studied yet. However the leaves are reputed to contain detergent agent (Sahle and Gashe, 1991).

2.3.2. *Weira* (*Olea europaea* L. *subsp* *cuspidate*)

Weira is commonly used in Ethiopia for smoking fermentation containers of traditional beverages such as *tella*, *tej*, containers of water, containers of milk and milk products the smoke is reported to remove and cause sluggish growth of coli forms in smoked milk and its products. It has been shown that *weira* smoke contains number of compounds which have the ability to kill or delay the rate of growth of a number of human pathogenic bacteria and fungi. *Weira* smoke give smoky flavor to water, *tella*, *tej* and milk (Mogessie *et al.*, 2001).

2.4. Fermentation

Fermentation in food processing is the conversion of carbohydrates to alcohol and carbon dioxide or organic acids using bacteria and yeast or both, under anaerobic condition. In fermentation, action of microorganism is desirable. During traditional fermentation substrates which may be of plant or animal origin, are converted into edible products by the physiological activities of microorganisms (Kumari *et al.*, 2015).

Fermentation is one of the ancient methods to preserve foods. Due to its nutritional value and variety of sensory attributes, it is popular in many cultures in which even today fermented

foods are part of the daily intake. In fact, fermentation is a relatively cost-effective, low-energy preservation process; it is essential to insure food shelf-life and safety. Moreover, it remains the major manufacturing technology for important food production (Clemencia *et al.*, 2014).

Foods and beverages arising from fermentation process continue to represent an important part of the global foodscape. Indeed, the Food and Agricultural Organization (FAO) of the United Nations noted the significance of the fermented products more than 15 years ago, highlighting their cultural and economic importance for local communities in the developing countries (Soukand, 2015).

Fermented cereals play a significant role in human nutrition in all parts of the world where cereals grow. Among all food fermentation (e.g., milk, meat, fish, vegetables and fruit), cereal fermentations reach the highest volume (Brandt, 2014).

The stored grains of cereals are metabolically in a resting state, which is controlled by the water activity ($a_w \leq 0.6$, 14% moisture). In this state the constituents are not available for microorganisms, and the endogenous enzymes are inactive. Fermentation process will be enabled under the influence of technological measures including addition of water, comminution by milling, and controlled management of microorganisms and enzyme activities. It's especially the addition of water that affects the ecological factors dramatically. After the water activity increases by water absorption, a reduction of the redox potential takes place by respiration, as well as the drop of pH by the respiration and fermentation. Substrates become available from endogenous hydrolytic activities (e.g. amylolysis, proteolysis and lipolysis) and physiological activities of deliberately added or contaminating microorganisms. These events cause continuous change of the ecological state in the cereal matrix (Walter *et al.*, 2005).

Natural cereal-based fermentations are usually induced by yeast, lactic acid bacteria and fungi, forming sometimes complex populations acting in concert. Yeast mainly degrades the carbohydrates, while bacteria show a proteolytic activity (Pandiella *et al.*, 2001). The basic cereal fermentation process involves the enzymatic activities of lactobacilli, leuconostoc, pediococci, yeast and moulds. Their metabolic activities result in the production of short chain

fatty acids such as lactic, acetic, butyric, formic and propionic acids. This reduces the pH to 4 or less (Kohajdova and Karovicova, 2007).

2.5. Cereal Based African Fermented Beverages

Indigenous fermented foods prepared from major cereals are common in many parts of Africa; some are used as alcoholic and some as non-alcoholic beverages (Ray and Didier, 2014).

Indigenous African beers are soured, fermented drinks made with malted sorghum, millet or maize. Identified by the grains used in their production, these beers are often called “sorghum,” “millet,” or “maize” beers. They are opaque and generally pinkish brown color due to the large quantity of solid particles and yeasts suspended in solutions. Because of the low alcohol content and large quantity of solids suspended in the beer, many consumers consider indigenous beer as much as food as a beverage. Regions of heavy indigenous beer consumption include a broad swath across the savannas of western Africa including northern regions of Ivory Coast, Ghana, Togo, Benin and Nigeria, virtually all of eastern and southern Africa, and the arid and highland areas of central Africa. Africa’s brewers confect a wide variety of indigenous beers using malt and a starchy adjunct like malted and unmalted sorghum, millet, or maize. The many possible combinations of malt and unmalted results in a wide assortment of indigenous beer recipes across the Africa continent (Steinkraus, 2004). Table 1 shows some of African indigenous beers, their local name and grains used for brewing.

Table 1. Some of African indigenous fermented beverages

Product Local Name	Substrates	Country
Aliha	Maize, sorghum	Ghana, Togo, Benin
Bushera	Millet	Uganda
Burukutu	Sorghum	Nigeria, Benin, Ghana
Bouza	Wheat	Egypt
Bogobe	Sorghum	Botswana
Chikokivana	Maize and finger millet	Zimbabwe
Doro	Finger millet malt	Zimbabwe
Kaffir beer	South Sudan	Kaffir corn
Keribo	Barley	Ethiopia
Maheu	Maize, sorghum, millet	South Africa
Mangisi	Millet	Zimbabwe
Mawe	Maize	Benin, Togo
Merissa	Sorghum and millet	Sudan
Otika	Sorghum	Nigeria
Pito	Maize, sorghum or a combination of both	Nigeria and Ghana
Seketeh	Maize	Nigeria
Talla	Maize, sorghum, wheat, barley, finger millet or a combination of them	Ethiopia
Ting	Sorghum	Botswana
Togwa	Maize, sorghum, millet or maize + sorghum	East Africa and Tanzania
Uji	Maize, sorghum, or millet	East Africa

Source: (Didier and Ray, 2014)

2.6. Shelf Stability of Traditional Beverages

Beer stability, defined as the ability of beer to maintain its properties unchanged from bottling to the end of estimated shelf life. Beer stability has three distinct aspects: biological, colloidal and sensory stability. Biological stability is related to the yeast and spoilage bacteria, which are possibly present in beer and could trigger undesirable sensory changes during shelf life. In order to ensure its biological stability, microorganisms have to be removed from beer, which for yeast cells is achieved by filtration. In colloidal stability the presence of visible haze can significantly limit the shelf life of beer. Haze formation is mostly attributed to proteins, polyphenols and their interactions (Pabby *et al.*, 2008). In order to extend the shelf life of beer, it can be either thermally pasteurized or subjected to a sterile micro porous filtration. Thermal pasteurization may affect the flavor of the beer. Micro-porous filtration can trap all microbes that are present in the beer, but may also remove a lot of the aroma, body, and even flavor (Cullen *et al.*, 2011).

Traditional fermentation practices are labor intensive, time consuming and craft-based resulting in lower productivity due to the factors like crude handling, lesser shelf life and lack of homogeneity. There are a variety of fermented products produced worldwide that have not received the scientific attention they deserve. The production of fermented beverages still remains today largely a traditional family art done at homes. There is no control in fermentation process. Shelf life of traditionally prepared drinks remains too low due to the presence of high microbial load as well as solid particle deposit which leads to off-flavors and turbidity, essentially due to spoilage (Shrivastava *et al.*, 2015).

Most traditional beverages have short shelf life and undergo rapid deterioration within 48 hours of production. This results in heavy losses being incurred by the local brewers since the unsold batches have to be discarded. Traditional beers spoil rapidly because they are relatively actively fermenting. Few attempts have been made to prolong the keeping quality of African traditional beers by pasteurizing, using preservatives and a combination of both (Sanni *et al.*, 1999).

2.7. Beer Spoilage Microorganisms

Micro-organisms exert indirect undesirable effects on brewing in three ways. Firstly, growth on raw material can produce undesirable changes such that the materials do not behave normally. Secondly, the growth of contaminants on raw materials can generate microbial metabolites, which can persist in to the brewing process and exert deleterious effects. Thirdly, very heavily contaminated raw materials can introduce microbial biomass that persists in to green beer. Although dead, the cells can cause beer filtration problems and even beer hazes if filtration is deficient (Briggs *et al.*, 2004).

Beer has been recognized for hundreds of years as a safe beverage. It is hard to spoil and has a remarkable microbiological stability. A few microorganisms still manage to grow in beer. These, so-called beer spoilage microorganisms, can cause an increase of turbidity and unpleasant sensory changes of beer. These changes can affect negatively not only the quality of final product, but also, the financial gain of the brewing companies (Rakcejeva *et al.*, 2013).

A number of microorganisms have been reported to be beer spoilage microorganisms, among which both Gram-positive and Gram-negative bacteria, as well as so-called wild yeasts. Gram-positive beer spoilage bacteria include lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus*. They are recognized as the most hazardous bacteria for breweries since these organisms are responsible for approximately 70% of the microbial beer-spoilage incidents. The second group of beer spoilage bacteria is Gram-negative bacteria of the genera *Pectinatus* and *Megasphaera*. The roles of these strictly anaerobic bacteria in beer spoilage have increased since the improved technology in modern breweries has resulted in significant reduction of oxygen content in the final products. Wild yeasts do cause less serious spoilage problem than bacteria but are considered a serious nuisance to brewers because of the difficulty to discriminate them from brewing yeasts (Sakamoto and Konings, 2003).

2.8. Commercial Beer Processing and Pasteurization

Beer is a fermented beverage brewed from malt and flavored with hops. Of all the herbs that have been used in beer, only the hop plant (*Humulus lupulus*) has gained widespread acceptance and is regarded as an essential raw material in the brewing industry, primarily for

its contribution to bitterness, which lends a more balanced and satiating palate to finished beer (Caballero *et al.*, 2012).

The basic ingredients in beer are water, malted cereals, hops and yeast. Water comprises 90-95 % of the content of finished beer, and its quality can influence the flavor of beer. Barley is the most common cereal used in the America, Europe and Ethiopia to produce malt, although small volumes of beer are made from other cereal grains. The conversion of cereals into beer is not a direct process. The cereals used in beer production do not contain sufficient quantities of fermentable sugar. These cereals must first undergo modification during the malting and mashing steps to yield carbohydrates that yeast can convert during the fermentation step into ethyl alcohol and carbon dioxide. Freshly produced beer can then be aged for flavor development before it undergoes finishing steps which can include filtering, pasteurizing, and packaging (Lederberge, 2000).

Pasteurization is the most common technique used to reduce the number of harmful microorganisms in beer. Two main types of pasteurizers are used: plate (flash) pasteurizers and tunnel pasteurizers. The latter are used mainly for in pack treatments. Flash pasteurization is used for continuous treatment of beer in bulk for subsequent fillings into kegs and occasionally for filling into sterile pack containers. The bacterial “kill” efficiency in pasteurization is determined both by the temperature (T , °C) and the time (t , minutes) for which the beer is held at that temperature. This is defined in terms of pasteurization units (PU) (Lea and Piggot, 2012). The commercial rule of thumb has been to use a PU of 15 min at 60 °C where 1 PU is define as exposure to 60 °C for 1 minute. A wide variance occurs in the temperature of pasteurization and numbers of PUs used among breweries. Although laboratory tests indicate that from 1 to 5PU are effective, 8 to 30 PU are generally used (Buzrul, 2006).

Pasteurization heat can be generated by hot water, dry heat or electric current and products are cooled promptly after heat treatment. Either the high temperature for a short time (HTST) or the low temperature long time (LTLT) can be used. In the processing of milk, beer and fruit juices by low temperature holding pasteurization, the liquid is maintained at 62.8 °C for 30 minutes. Products can also be held at 71 °C for 15 seconds. These treatments are equivalent

and sufficient to destroy the most heat resistant non-spore forming pathogenic organisms (Khan, 2015).

3. MATERIALS AND METHODS

3.1. Survey (Phase One)

3.1.1. Study site

The study was conducted in Jimma town, in 5 selected kebelles namely. Table 2 summarizes the geographical and demographic characteristics of the study area.

Table 2. Geographical and demographic description of the study area

Description	Jimma town
Location	Oromia National Regional State, Jimma zone, Ethiopia 356 km Southwest of Addis Ababa
Coordinates	7° 4' North Latitude and 36° 5' East Longitude
Elevation(m)	1725–1789
Mean annual rainfall(mm)	1470
Mean annual Temperature(°C)	19.15
Population	120,960

Source: Ministry of Urban development and housing website

<http://www.mwud.gov.et/web/jimma/home> , accessed on June 7/2016

3.1.2. Study design and sampling

The study used a cross-sectional survey design and data were collected from 66 local *tella* makers. Five *kebelles* (*Bosa kito*, *Bosa addis*, *Merkato hermata*, *Merkato menahariya* and *Becho bore*) were selected purposively. These kebelles have more *tella* makers compared to other kebelles in Jimma town. Since there is no any data available regarding the total number of *tella* makers at the regional statistics office, a door to door interview was carried out.

3.1.3. Time of the study

The survey was carried out in May, 2014.

3.1.4. Inclusion criteria

The study included respondents who made *tella* for sell at household level.

3.1.5. Data collected

The study employed both qualitative and quantitative data collection methods. The questionnaires were initially prepared in English language and later translated into Amharic. A pre-test of the interviews were carried out before the actual interviews were started. Data were collected by the study researcher using semi structured questionnaire. The questionnaire was divided in to three parts; Socio demographic, preparation and making of *tella* and problem related to *tella* making.

The socio demographic part included the following information. Gender, age, marital status, educational status, religion and house hold size. The second section included information related to preparation and making of *tella* such as experience of the respondents, what kind of beverage they prepare/sale other than *tella*, time needed for tella making, batches of tella made, type and amount of ingredients, materials used for making *tella*, shelf stability of tella and source of energy used. The last section consisted of questions related to challenges faced by tella makers.

3.1.6. Data analysis

The collected data was sorted then analyzed using statistical package for social science software (Version 20 IBM Inc, Chicago, IL, USA).

3.2. Study of Effect of Ingredients on Quality of *Tella* (Phase Two)

3.2.1. Description of the study area

The experiment was conducted in Jimma University College of Agriculture and Veterinary Medicine Postharvest Management laboratory. Jimma University College of Agriculture and Veterinary Medicine is geographically located at south, at 7°33' N and 36° 57' E longitude at an altitude of 1710 meters above sea level Southwest of Addis Ababa (*BPEDORS, 2000*).

3.2.2. Experimental design

The experiment was a 3x3x3 factorial experiment with 3 replication having a total number of 81 treatments. Table 3 shows factors and their level. The experiment was done to identify the effect of different malt and adjunct combination and fermentation duration on the physicochemical properties of *tella* in order to select the best treatment combination for the shelf life study.

Table 3. Factors and their levels

Factors	Levels
Factor 1 = Malt combination	1. Wheat, barley & maize (1:1:1) 2. Wheat, barley, maize & millet (1:1:1:1) 3. Wheat & barley(1:1)
Factor 2= Adjunct combination	1. <i>Enikuro</i> 2. <i>Yetelakita & enikuro(1:1)</i> 3. <i>Yetelakita, enikuro& asharo (1:1:1)</i>
Factor 3 = Fermentation duration	1. One day 2. Two days 3. Three days

Model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijk} \dots \dots \dots \text{Equation 1}$$

Where,

X_{ij} = measurement for all observation

μ =over all mean

α_i =effect of the i^{th} level of the first factor

β_j =effect of the j^{th} level of the second factor

γ_k =effect of the k^{th} level of the third factor

$(\alpha\beta)_{ijk}$ =interaction between the i^{th} level of the first factor, the j^{th} level of the second factor and the the k^{th} level of the third factor

ε_{ijk} =experimental error

3.2.3. Experimental materials and preparation

Plastic buckets, sacks, trays, plastic sheets, baking pan (*birret mitad*), disk miller (Kalkob, D6072 Dreich, West Germany) were used during the preparation of ingredients of *tella*. Plastic buckets were used as a fermentation tank. Each bucket was cleaned using 200 grams of *grawa* (*Vernonia amygdalina Del*) leaves rinsed with water and finally smoked using dried *weira* stem (*Olea europaea L. subsp cuspidate*). This is commonly done to use the smoke as a means to disinfect the utensils and to impart good flavor on *tella*.

Ingredients used for preparation of *tella* were *Gesho* (*Rhamnus prinioides*) local hops, maize, barley, wheat and millet which were purchased from Jimma local market. All of the ingredients were cleaned and impurities like stones and dusts were removed by hand and sieving.

3.2.3.1. Gesho (*Rhamnus prinioides*) preparation

Both *Gesho* leaves and stems were sundried, pounded using wooden mortar and pestle and packed in a polyethylene bags. Tap water was used to brew *tella*. Figure 1 shows *gesho* (*Rhamnus prinioides*) (A) Sundried leaves (B) Pounded leaves (C) Dried stem (D) Pounded stem which were prepared to make *tella*.





Figure 1. *Gesho*(*Rhamnus prinioides*) (A). Sundried leaves (B). Pounded leaves (C). Sundried stem (D). Pounded stem

3.2.3.2. Malt preparation

Malt was prepared from wheat, barley, finger millet and maize. These four cereals for malting were selected based on the result of the survey.

Wheat and barley grains were cleaned by removing defective grains, dust, stones and other extraneous materials. Then grains were steeped in water with a volume two times the weight of the grain for 24 hours at room temperature. The water was drained off, grains were wrapped with sack and allowed to germinate for 48 hours. Germinated grains were sun dried until the moisture content reduced to 13 % then milled using a disk miller (VDE 0530/84, West Germany). Figure 2 shows malted barley (A) and wheat (B).

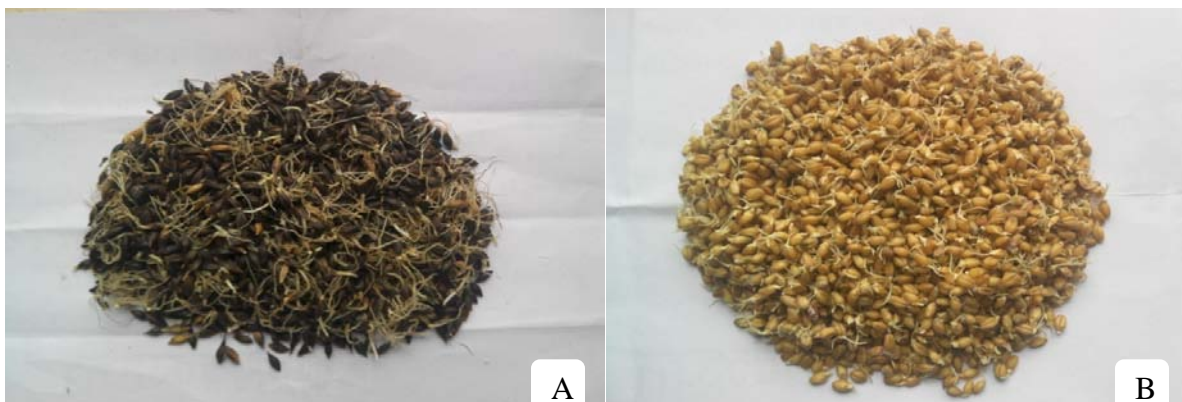


Figure 2. Malt (A). Barley (B). Wheat

Cleaned finger millet grains were washed, drained, steeped in water for 24 hours, drained and allowed to germinate for 48 hours. Then sun dried (13%) and milled using disk miller (VDE 0530/84, West Germany) (Figure 3).



Figure 3. Finger millet malt

Cleaned maize was steeped for 48 hours, rinsed twice, steeped again for 24 hours, rinsed and let to germinate for 72 hours wrapped with sack (Figure 4). It was sun dried until the moisture dropped to 14% and milled using a disk miller (VDE 0530/84, West Germany).



Figure 4. Maize malt

3.2.3.3. Preparation of adjuncts

I. *Yetella kita* (flat bread)

Yetella kita was prepared from maize flour. Maize grains were cleaned and milled using disk miller (VDE 0530/84, West Germany). Dough was prepared by mixing maize flour and water, mixed well with hands then fermented for 12 hours. *Yetella kitta* was baked using a metal griddle pan (*biretmitad*). The baked flat bread was broken in to pieces and left to cool to room temperature.

II. *Enkuro*

Enkuro was prepared from maize flour by moistening it with water and then was roasted using a metal griddle pan (*biretmitad*) until color turns nearly to brown.

III. *Asharo*

Barley was roasted using baking pan until the color turns dark brown. The roasted barley was allowed to cool to room temperature and was milled using disk miller (VDE 0530/84, West Germany).

3.2.3.4. *Tella* making

Tella was brewed in Postharvest laboratory of Jimma University College of Agriculture and Veterinary Medicine. Treatments were randomized; each bucket was labeled accordingly and arranged based on the randomized layout away from each sides of the wall. *Tella* was made as follows. Buckets were smoked over smoldering *woira* stems. One hundred twenty five grams of pounded gesho leaves were mixed with one liter of water and allowed to ferment for three days. Two hundred grams of malt (three malt types in to separate buckets), 150 grams of pounded gesho stem were added and mixed using one liter of water. Adjuncts like *enkuro* (1kg), *yetella kita* (1kg) and *asharo* (1kg) were added as per the treatment, mixed by adding one liter of water and then allowed to ferment for two days. Three liters of water was added to dilute *difdif* and allowed to ferment for one, two and three days (three different fermentation times). *Tella* was filtered using cheesecloth and kept in clean buckets for analysis.

3.2.4. Data collected

A. pH

pH of *tella* was determined by dipping an electrode of a digital pH meter (HI-98129, Mauricious) in to a beaker containing 50 ml of *tella* sample. The pH meter was calibrated against standard buffers of pH 4 and 7 (AOAC 2005, method 945.1).

B. Total soluble solids (TSS)

TSS was determined using hand held refractometer (DR201-95, Germany). A drop of *tella* was placed on the prism of the refractometer using a pipette and the reading was recorded as °Brix (Muyanja and Namugumya, 2009).

C. Titratable acidity (TA)

TA was determined according to AOAC (2005) method 950.07. 250mL of H₂O was boiled for 2 minute. From fast-flowing pipette, 25 ml of *tella* which was previously decarbonated by shaking and filtering was added and heated for 60s, regulating heat so that solution resumes boiling during 30 s, removed from heat, stirred and cooled to room temperature. Then 0.5 ml of 0.5 % phenolphthalein solution was added and titrated with 0.1M NaOH. It was titrated to first appearance of faint pink and burette reading was recorded. The burette reading was taken as end point and the percent of lactic acid present in the sample was calculated using Equation 2. The result was reported as lactic acid equivalent to the nearest 0.01 % (1ml 0.1M alkali=0.009 g lactic acid).

$$Lacticacid(\%) = \frac{AmountofNaOH \times NormalityofNaOH \times 0.009}{Volumeofsample (mL)} \dots\dots\dots Equation 2$$

3.2.5. Data analysis

Data was subjected to analysis of variance using statistical analysis software (version 9.2, SAS Inc, Cary, NC, USA). Prior to analysis data was checked for normality. Multiple comparisons among treatment means were done using Tukey method at P<0.05.

acquisition unit (Agilent 34970A, Malaysia) to monitor and record time-temperature history of the samples during heating and cooling phases. Pasteurization unit (PU) commonly used in beer factories was used as reference processing condition (1 PU=1 min heating at 60 °C) to determine sufficiency of pasteurization condition. The viscometer bath temperature was set at 60 °C and 65 °C for two different trials and the change in temperature was recorded every minute interval until the temperature at the slowest heating point reaches these temperature.

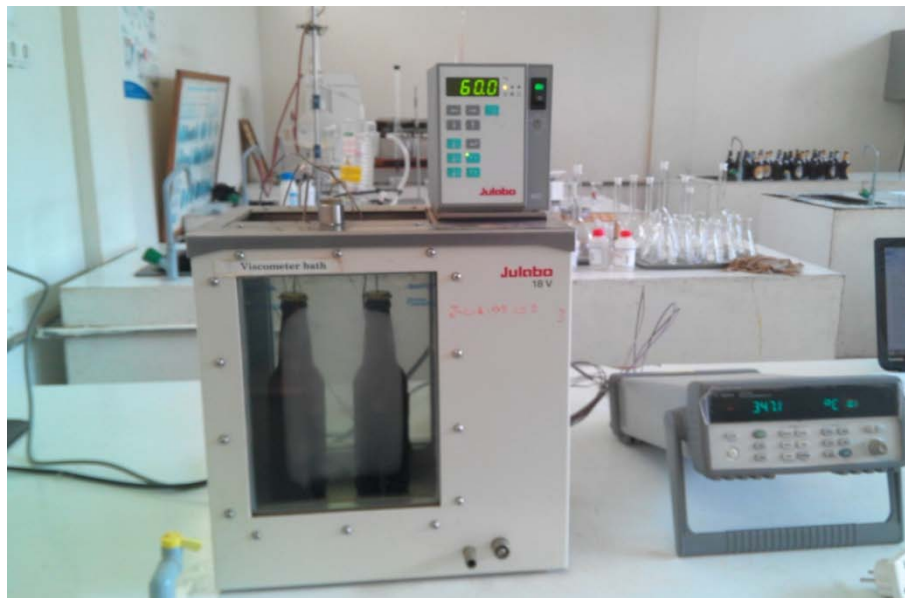


Figure 5. A viscometer bath coupled with a data acquisition unit to gather time-temperature data during heating of bottles filled with *tella*

II. Pasteurization Unit (PU)/ Pasteurization Equivalent (PE)

To determine required PU /PE, lactic acid bacteria was considered as a target bacterium since it is the main spoilage bacterium in beer industries. Destruction of the bacterium during heating is a time dependent process. PU/PE provides the temperature influences sum on the microorganism evolution in production. In order to destroy all vegetative cells a unit of pasteurization is required as a minimum requirement. One unit of pasteurization (PU) represents destruction of lactic acid bacteria for one minute at 60°C. PUs at each temperature level was calculated using Equation 4.

$$PU = t * 10^{\left(\frac{T-T_{ref}}{z}\right)} \dots\dots\dots \text{Equation 4}$$

Where,

PU = Pasteurization Unit (min)

t = holding time (min)

T = effective holding temperature ($^{\circ}\text{C}$) at time t

T_{ref} = reference temperature (60°C)

z = temperature coefficient of lactic acid bacteria (6.94°C)

The minimum PU should be 5 min, but for this study purpose considering high degree of lactic acid contamination, for 60°C a total PU time of 15.86 min and for 65°C a total PU of 7.79 min were designed and applied (Appendix Figure 1 and 2).

III. *Tella* making

Buckets were smoked over smoldering *woira* stems. 135 grams of pounded gesho leaves was mixed with one liters of water to make *tinsis*. It was allowed to ferment for three days. 320 grams of malt, 2 kg of *enkuro* and 300 grams of pounded gesho stem was added to make *difdif*. Two liters of water was added. The *difdif* was allowed to ferment for 2 days. Four liters of water was added to dilute *difdif* and allowed to ferment for two days. *Tella* was filtered using cheesecloth and transferred in to clean buckets and made ready for analysis and bottling.

IV. Bottling and pasteurization of *tella*

Beer bottles and crown corks were cleaned with detergent and boiled in a water bath. For control treatments unpasteurized *tella* was bottled and capped. Bottled *tella* was pasteurized at 60°C for 23 min (13 min come up time and 10 min holding) to achieve PU of 15.86 min and at 65°C for 17 minutes (7 min come up time and 10 min holding) in a viscometer bath to achieve PU of 7.79 min and allowed to cool by immersing the bottles in a bucket of water which was at room temperature. Bottles were labeled and placed on table to study change on parameters properties and their storage stability (Figure 6).



Figure 6. Bottled *tella* samples laid out in a complete randomized design (CRD) after they were pasteurized

V. Shelf stability study

Extended shelf stability study method was used to determine the shelf life of *tella*. Bottled samples were arranged on tables away from direct sunlight and heat source for three months. Microbial (Total Aerobic Count (TAC)) and physicochemical analysis were done every 10 days during the first month, every 15 days interval on the second month and once at end of the third month. The average room temperature and relative humidity during the storage period was 23 °C and 65.5% respectively. The shelf-life study was carried from August to October 2015.

3.3.3. Data collected

A. pH

pH of *tella* was determined by dipping an electrode of a digital pH meter (HI-98129, Mauricious). The pH meter was calibrated against standard buffers of pH 4 and 7 (AOAC 2005, method 945.1).

B. Total soluble solids (TSS)

TSS was determined using hand held refractometer (DR201-95, Germany). A drop of *tella* was placed on the prism of the refractometer using a pipette and the reading was recorded as °Brix (Muyanja and Namugumya, 2009).

C. Titratable acidity (TA)

TA was determined according to AOAC (2005) method 950.07. 250mL of H₂O was boiled for 2 minute. From fast-flowing pipette, 25 ml of *tella* which was previously decarbonated by shaking and filtering was added and heated for 60s, regulating heat so that solution resumes boiling during 30 s, removed from heat, stirred and cooled to room temperature. Then 0.5 ml of 0.5 % phenolphthalein solution was added and titrated with 0.1M NaOH. It was titrated to first appearance of faint pink and burette reading was recorded. The burette reading was taken as end point and the percent of lactic acid present in the sample was calculated using Equation 5. The result was reported as lactic acid equivalent to the nearest 0.01 % (1ml 0.1M alkali=0.009 g lactic acid).

$$Lacticacid(\%) = \frac{AmountofNaOH \times NormalityofNaOH \times 0.009}{Volumeofsample (ml)} \dots\dots\dots Equation 5$$

D. Alcohol content

Alcohol content was measured using hydrometer. One litter of *tella* was poured in to a measuring cylinder and hydrometer was immersed. The reading was recorded as percentage (Gizaw, 2006).

E. Sensory evaluation

Sensory evaluation of samples was made only for one time immediately after pasteurization process (day 1) in order to evaluate the effect of pasteurization on sensorial properties of samples. Due to extended storage time, ethical issues and absence of volunteers and sensorial properties of samples for different storage period were not evaluated during the storage period.

Sensory evaluation was done using 30 untrained panelists using 5point hedonic scale (1= dislike extremely, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = like extremely) to assess the sensory attributes such as aroma, color, mouth feel and overall acceptability. A separate sensory evaluation sheet was developed for taste and flavor in order to specify the preference of panelists. Taste had four categories namely sweet, bland, sour and bitter coded as 1, 2, 3 and 4 respectively. Except for bitterness the three terms are used to refer off tastes which are the result of under fermentation, loss of taste components and over fermentation. Flavor was categorized in to three namely yeasty, fruity and hoppy coded as 1, 2, and 3 respectively. Yeasty and fruity are off-flavors caused by inefficient inactivation of yeast during pasteurization and inadequate fermentation respectively. A separate sensory sheet was prepared based on beer sensory evaluation used by breweries. This descriptive terms used for taste and flavor help to identify between good taste and flavor attributes from off flavor.

F. Microbial analysis

I. Media preparation

Thirsty three grams of de Man Rogosa and Sharpe agar (MRS agar) was added in to a volumetric flask containing 250 ml of distilled water. The mixture was stirred using a magnetic stirrer on a hot plate and boiled for 15 minutes which was later sterilized at 120 °C for 30 minutes using autoclave (KMA 240N, England).

II. Sample preparation and plating

Tella samples were opened and transferred into test tubes under aseptic conditions to conduct Aerobic Plate Count (APC). Aliquots (10 ml) of *tella* samples were serially diluted in 90 ml of sterile distilled water and vortexed for 10 seconds. Then 0.1 ml of each dilution was streaked on to de Man Rogosa and Sharpe agar (MRS agar) plates using a bent glass rod and incubated for three days at 30 °C. Following incubation the number of colonies was counted and colony forming units (CFU/ml) were calculated (Garafalo *et al.*, 2015).

3.3.4. Data analysis

Data was subjected to analysis of variance using statistical analysis software (version 9.2, SAS Inc, Cary, NC, USA). Prior to analysis data was checked for normality. Multiple comparisons among treatment means were done using Tukey method at $P < 0.05$.

4. RESULTS AND DISCUSION

4.1. Survey (Phase One)

4.1.1. Socio-demographic characteristics of respondents

The highest number of respondents was from *Kebele Becho bore* and the lowest from *Merkato menahariya*. Table 4 shows socio-demographic characteristics of respondents.

Table 4. Socio-demographic characteristics of respondents in selected *kebeles* of Jimma town, May, 2014, N=66

Variables	Catagories	ns	(%)
<i>Kebeles</i>	<i>Bosa kito</i>	14	21.2
	<i>Bosa addis</i>	14	21.2
	<i>Merkato hermata</i>	12	18.2
	<i>Merkato menahariya</i>	11	16.7
	<i>Becho bore</i>	15	22.7
Gender	Male	0	0
	Female	66	100
Age group	<20	1	1.5
	21-30	11	16.7
	31-40	20	30.3
	41-50	15	22.7
	>50	19	28.8
Marital status	Single	5	7.6
	Married	36	54.5
	Divorced	9	13.6
	Widowed	16	24.2
Educational status	Formal education	44	66.7
	Informal education	22	33.3

Table 4. Continued

	2	9	13.6
House hold size	3-6	40	60.6
	7-10	15	22.7
	>10	2	3
Brewing skill sources	Mothers	62	93.9
	Sisters	1	1.5
	Neighbors	3	4.5
Work experience(years)	<2	4	6.1
	3-6	21	31.8
	7-10	7	10.6
	>10	34	51.5

In all the 5 kebeles, 100% of the respondents were women. Most *tella* preparation activities were performed by women. In some cases, male family members take part in preparing firewood, fetching water and sometimes serving customers. The largest proportion of the survey population was in the age range of 31-40. The population data split by marital status showed that 54.5% were married, 24.2% widowed, 13.6% divorced and 5% were single. From the 66 respondents, 33% were informally educated while the rest 66.7 percent were formally educated. Majority of the respondents had a family size of 3-6, 14% had 2 and only 3% had a family size greater than ten.

Tella being a very popular traditional alcoholic drink in Ethiopia, its recipe passes from generation to generations mainly through female family members in the household. A very evident finding was that almost 62% of the respondents learned its preparation from their mothers, only 1 % from their sisters and 3% from their neighbors. With regards to the length of time the respondents have been making and selling *tella*, most have been in the business for more than 10 years which counts for 51.5 % of the respondents and 31.8 % have been making *tella* for 3-6 years, 10.6% for 7-10 years and 6.1% for less than 2 years.

Some of the respondents sell additional alcoholic drinks such as *areque* (a distilled alcoholic drink). Seventy nine percent of the respondents sell only *tella* and the rest 21 % sell both *tella* and *areque*. In most *tella* vending houses snacks like *kolo* (roasted cereals and legumes) and *bekolt* (germinated bean and chickpea) were served with a hot sauce which is prepared from mashed hot pepper and spices which customers have to pay extra for these snacks.

4.1.2. Equipments used for making *tella*

Tella brewers use different equipments to prepare *tella*. Plastic sheets and sacks were used to dry and store grains. A circular traditional tray (*sefed*) and sieve were used to clean grains. Wooden mortar and pestle (*mukecha*) were used to pound *gesho* (*Rhamnus prinoides*). Disk miller was used to grind grains although some brewers prefer to use traditional grinding stone (*ye dingay wefcho*) to grind malt. Metal griddle (*biret mitad*) was used to bake *yetella kita* and roast grains. Plastic buckets and barrels were used to make and store *tella*. Some also use metal barrels. Glasses, tin cans, plastic cups were used to serve *tella*.

4.1.3. Source of energy

Tella making involves a number of roasting and baking. Thus brewers use different sources of energy. Fire wood (81.5%), saw dust (10%), coffee husk (5.2%), cow dung (3.3%) were commonly used sources of energy. Sun drying was also used to dry grains, *gesho* and firewood.

4.1.4. Ingredients of *tella*

Tella is prepared from *gesho* (*Rhamnus prinoides*), wheat (*Triticum sativum*), barley (*Hordeum vulgare*), maize (*Zea mays*), finger millet (*Eleusine coracana*) and water. Wheat, barley, maize and finger millet were used as malted and unmalted ingredients.

Gesho is what gives *tella* the bitter characteristic and desired flavor. Most respondents prefer buying dried *gesho* from the local market, clean, pound and make it ready for use. Whereas others buy fresh *gesho* and prepare dried powder at home. *Gesho* leaves and stems are sun dried, leaves are shaken off the stems, cleaned and sorted out. *Gesho* seeds are carefully picked-out since it is believed to lower the quality of *tella* by giving it undesirable flavor.

Dried leaves and stems are pounded separately using a traditional wooden mortar and pestle (*mukecha*).

Malt is another ingredient which serves as source of enzyme amylase which is needed to convert starch in to simple sugars. It can be prepared from barley, wheat, maize and finger millet. To prepare malt, defective grains and extraneous matters are cleaned first. Grains are soaked overnight to days until they have absorbed enough water for germination. The length of soaking differs from grain to grain. Wheat, barley and finger millet are soaked for a day while maize is soaked for two or three days with a thorough rinsing in between consecutive days. After the water has been drained well, it is wrapped with plastic sheets or sacks and sometimes with *Enset* (*Enset ventricosum*) leaves and left to germinate. Some respondents unwrap and sprinkle water to make sure it is wet enough to germinate. Wheat, barley, finger millet take two days or less to germinate and maize takes three days. Mostly malt is sun dried but some prefer to dry it by hanging the malt on traditional kitchen walls. Germinated grains are spread over plastic sheets, plastic mats or sacks and left to dry till the moisture drops to the point where the grains become dry enough to be milled. Most respondents use nearby disk mills while few use traditional grinding stones to grind malt. Malt flour eventually is stored in plastic bags or sacks and stored in cool and dry place.

Malt preparation can take from six to ten days. During the rainy season drying alone can take more than a week. Given the long time and effort needed to prepare malt, only 11% prepare malt at their home. The rest 90 % buy prepared malt from the local market. The type of cereal used, the amount and the proportion of mixing ingredients differed from respondent to respondent. Most brewers prefer mixing different type of cereals rather than using a single type of grain. The choice of ingredients was affected by the personal preference of the brewers, availability and price of ingredients. Type of cereal used for malt by the respondents are listed in Table 5.

Table 5. Types of cereals used for malting by the respondents

Type of malt	n	%
Wheat & barley	7	10.6
Wheat & maize	3	4.5
Finger millet & maize	1	1.5
Wheat, barley & maize	30	45.5
Wheat, barley, maize & finger millet	22	33.3
Only one type of cereal	3	4.5
Total(N)	66	100

Yetella kita, *enkuro* and *asharo* are adjuncts which are prepared from unmalted cereals flour like barley, maize, millet and sorghum. *Yetella kita* is flat bread prepared from mix of maize, barley, sorghum and millet or only from a single type of cereal. The preferred cereal is milled, mixed with water to prepare the batter then baked on a metal griddle. *Enkuro* is prepared from flours of maize and barley. The flour is moistened with water until it is wet enough to form a consistent texture then toasted using a metal griddle (*biretmitad*) until the color turns nearly brown. Barley is roasted first to prepare *asharo* and then milled. *Asharo* doesn't need further cooking. Almost 80% of the respondents use only *enkuro* while the rest use combination of *enkuro* and *yetella kita*. It is believed that using combination of additional substrates imparts sourness and shortens shelf stability of *tella*. Cost wise, using one type of adjunct lowers production cost and time needed for preparation of *tella*.

4.1.5. *Tella* preparation steps

The length of time needed to prepare *tella* ranged from six to nine days. But 73% of the respondents reported that it takes them seven days to prepare *tella*. This doesn't include the time needed to prepare and make ready all the ingredients. The length of time needed to prepare *tella* varies from respondent to respondent. This is mainly because the length of each fermentation phase it passes through depends on their recipe and experience. Most do their best to give their brew a good finish and flavor.

A plastic bucket, wooden or plastic barrel is used as a fermentation vessel. Container is cleaned using *girawa* (*Vernonia amygdalina Del*) until it forms foams and rinsed with water then smoked with *Woirra* (*Olea africana*) wood for minutes. These activities are mainly done

to control possibility of microbial contamination and spoilage and to impart good flavor respectively. Pounded *gesho* and water are added to the container then mixed well and left to ferment for days. This marks the first phase of fermentation locally known as *tinsis*. This fermentation can be as short as one day (1%). Some respondents ferment it for four days (16.7%) and even longer depending on the ambient temperature condition. However, the majority of respondents (74%) ferment *tinsis* for three days (Table 6).

Table 6. *Tinsis* fermentation duration reported by the respondents

<i>Tinsis</i> fermentation duration (days)	N	(%)
1	1	1.5
2	3	4.5
3	49	74.2
4	11	16.7
>5	2	3

The second phase fermentation starts when malt is added along with adjuncts like *enkuro*, *yetella kita* and *asharo* to form a thick blend of ingredients known as *difdif*. Most of the respondents only add *enkuro* while some add mix of *yetella kita* and *enkuro*. Only few add *enkuro*, *yetella kita* and *asharo* on different days. *Difdif* is fermented from one to three days depending on the preference of the brewers. Thirty six percent of the respondents reported that they ferment *difdif* for 2 days. At this stage some malt might be added to speed up the fermentation if necessary.

The third fermentation phase commence once after *difdif* is diluted with water and mixed well. The fermentation can take only a day or two. Two different layers can be clearly seen at this final stage. The thick sediment at the bottom which is the spent of *tella* known as *atela* and a relatively clearer and light colored liquid at the top (*tella*). This phase gives the final beverage *tella*. To maintain the flavor and the keeping quality, it is carefully poured to another clean container. Figure 7 summarizes the steps in preparation of *tella*.

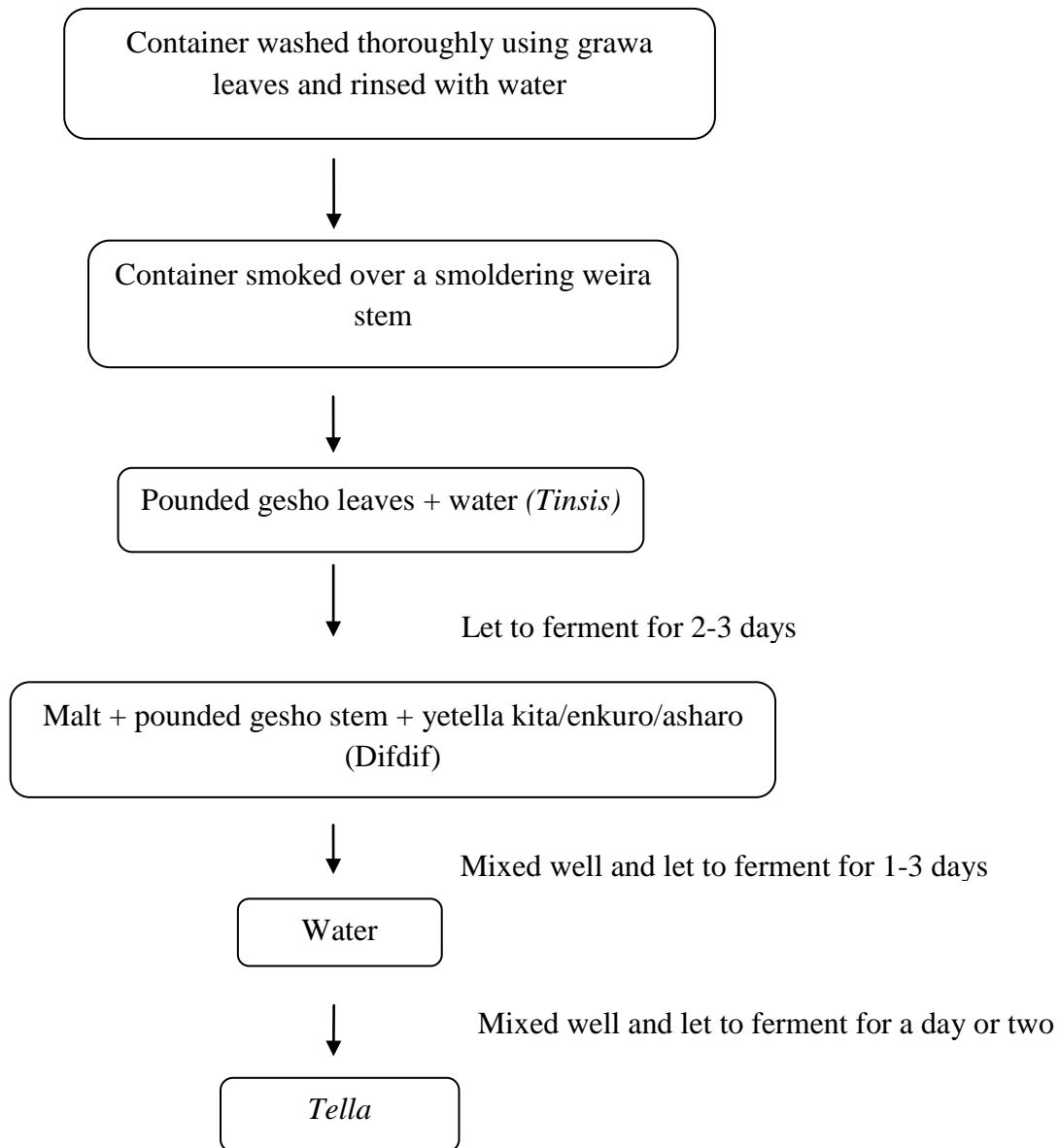


Figure 7. Flow diagram of *tella* preparation

4.1.6. Shelf stability of *tella* reported by the respondents

The shelf life of *tella* appears to be very short. Respondents were asked for how long their *tella* can be kept without a noticeable flavor change is observed. Forty six percent of the respondents reported that they can keep *tella* for four days, 41 % for three days and 6% of them for 5 days. The shortest shelf life was for 2 days as reported by 5% of the respondents

(Table 7). Cleaning containers thoroughly, using a good quality *gesho* and malt, cleaning grains well, cooking ingredients to the right point and cooling them before adding, checking whether fermentation is taking place properly or not, keeping heat sources away and personal hygiene are some of the routines listed by the respondents in order to prepare a good quality *tella* with a better shelf life.

Table 7. Shelf stability of *tella* as reported by the respondents, N=66

Shelf stability of <i>tella</i> (days)	n	(%)
2	3	4.5
3	27	40.9
4	30	45.5
5	4	6.1
>5	2	3

4.1.7. Challenges encountered by *tella* vendors

Basically, unstable price of ingredients was the major problem mentioned by most respondents. Whenever price of ingredients goes up they cut their production by half or discontinue making *tella* for some time until the price goes down which had them earn a very low and undependable income. Also seasons and customer demand affect their income. *Tella* demand increases during summer. Since it's hot, *tella* is used as a thirst quencher among most of middle and low income communities. In the contrary, the demand is very low during winter due to the cold weather. Thus their income greatly differs throughout the year.

Unsuitable working condition was the second problem. The living condition of the respondents clearly revealed their problems. Most live in rented small houses which are too small to accommodate their families and customers at a time. *Tella* brewing requires manual labor. Consequently respondents spend long working hours in the kitchen and outdoors preparing ingredients. These conditions have put them in a situation where they couldn't take care of themselves resulting in a recurring health problems and related medical expenses.

Lack of support from financial institutions to this business was another challenge. As most of the respondents mentioned financial institutes support formal business sectors rather than informal businesses like local *tella* vendors.

Some *tella* vendors mentioned they were victims of verbal abuse and sexual harassment. Some drunken customers refuse to pay or get in to fights which in some cases end up being a serious conflict.

4.2. Effect of Malt, Adjunct Combination and Fermentation Time on Physicochemical Properties of *Tella* (Phase Two)

4.2.1. pH

Malt combination and fermentation duration had a significant effect on pH ($P < 0.05$) (Appendix Table 1). As indicated in Table 8, the pH ranged from 4.29 to 4.61. A study made by Fekadu *et al.* (2013) on the physicochemical properties of *tella* in 5 areas of Jimma town reported a similar result. In their study pH values of *tella* ranged from 4 to 4.8. The highest pH value 4.61 was recorded for *tella* brewed using a combination of *enkuro* and *yetella kita* and fermented for one day. *Tella* brewed from a combination of *enkuro* and *yetella kita* and fermented for two days had the lowest pH 4.29. This result is in line with Muyanja *et al.* (2003) who reported the pH of *Bushera*, an Ugandan fermented beverage prepared from a mixture of millet and sorghum had the lowest pH values between 3.7 and 3.8, whereas sorghum *bushera* had the highest pH value from 7 to 4. The progressive fall in pH and rise in titratable acidity that occurs during fermentation is characteristic of fermenting cereal grains (Singh *et al.*, 2003).

Table 8. Interaction effect of malt combination and fermentation duration on pH

Malt combination	Fermentation time(days)	pH
Wheat + barley + maize	1	4.59 ^a
	2	4.53 ^a
	3	4.41 ^{ab}
Wheat + barley + maize + finger millet	1	4.61 ^a
	2	4.29 ^b
	3	4.43 ^{ab}
Wheat + barley	1	4.58 ^a
	2	4.54 ^a
	3	4.42 ^{ab}
SE		0.05
P-value		0.029
CV (%)		3.39

Mean values followed by the same letter in a column are not significantly different at $P < 0.05$
Wheat + barley + maize (33% w/w) Wheat + barley + maize + millet (25% w/w) Wheat + barley (50% w/w)

4.2.2. TA

TA was significantly affected by adjunct combination ($P < 0.05$) (Appendix Table 3). *Tella* brewed using combination of three adjuncts Enkuro, yetella kita and asharo has a TA of 0.31 which was the highest. The lowest TA was obtained from tella brewed using only one type of substrate, enkuro (Table 9). This could be due to the added starch sources which directly contribute to the availability fermentable sugars which later are metabolized in to organic acids such as lactic acid which increases the TA.

Table 9. Effect of adjunct combination on TA

Adjunct combination	TA
<i>Enkuro</i>	0.26 ^b
<i>Enkuro & yetella kita</i>	0.28 ^{ab}
<i>Enkuro, yetella kita and asharo</i>	0.31 ^a
SE	0.009
P-value	0.005
CV (%)	16.2

Mean values followed by the same letter are not significantly different at $P < 0.05$

Enkuro (100% w/w), *Enkuro + Yetella kita* (50% w/w), *Enkuro + Yetella kita + Asharo* (33.3% w/w)

4.2.3. TSS

As indicated in Table 10, the interaction effect of malt combination and fermentation duration showed a significant effect on TSS ($P < 0.05$) (Appendix Table 2). The highest TSS 4.25 was recorded for *tella* brewed from a combination of wheat, barley and maize malt fermented for two days. TSS of samples increased at the second day and dropped at the third day for all malt types. The TSS decreased with fermentation time probably due to mainly microbes that acted upon the sugars to produce lactic acid and alcohol while they are deriving energy from the sugars (Parawira *et al.*, 2012).

Table 10. Interaction effect of malt type and fermentation time on TSS

Malt combination	Fermentation time(days)	TSS
Wheat + barley + maize	1	4.21 ^a
	2	4.25 ^a
	3	3.47 ^d
Wheat + barley + maize + finger millet	1	4.08 ^{ab}
	2	4.2 ^a
	3	3.9 ^{abc}
Wheat + barley	1	3.75 ^{bcd}
	2	4.14 ^a
	3	3.67 ^{cd}
SE		0.084
P-value		0.001
CV (%)		6.7

Mean values followed by the same letter are not significantly different at $P < 0.05$

Wheat + barley + maize (33% w/w) Wheat + barley + maize + millet (25% w/w) Wheat + barley (50% w/w)

Total soluble solid was significantly affected by the interaction of adjunct combination and fermentation time ($P < 0.05$). The mean TSS was 3.9 ± 0.08 . *Tella* brewed using adjunct combination of *enkuro*, *yetella kita* and *asharo* fermented for two days has the highest TSS 4.9 among the rest of the treatments. These three adjuncts serve as additional source of starch for the fermentation process hence contributing to the high TSS. The lowest TSS was 3.4 for *tella* brewed from only *enkuro* fermented for one day (Table 10). Generally, as stated in Tanguler (2014), type and amount of cereal used, fermentation time and temperature are most important factors which affect physicochemical properties of cereal based fermented beverages.

Table 10. Interaction effect adjunct combination and fermentation time on TSS

Adjunct combination	Fermentation time(days)	TSS
<i>Enkuro</i>	1	3.4 ^d
	2	3.98 ^b
	3	3.53 ^{cd}
<i>Enkuro + yetella kita</i>	1	3.88 ^{bc}
	2	3.7 ^{bcd}
	3	3.82 ^{bcd}
<i>Enkuro + yetella kita + asharo</i>	1	3.88 ^{bc}
	2	4.91 ^a
	3	3.72 ^{bcd}
SE		0.084
P-value		<.0001
CV (%)		6.7

Mean values followed by the same letter are not significantly different at P<0.05

Enkuro (100%w/w) *Enkuro + Yetella kita* (50% w/w) *Enkuro + Yetella kita + Asharo* (33.3% w/w)

TSS was also significantly affected by the interaction of malt and adjunct combination (P<0.05) (Appendix table 2). Malt combination of wheat, barley, maize and finger millet and substrate combination of enkuro, yetella kita and asharo gave the highest TSS 4.85. The lowest TSS 3.56 was recorded from *tella* brewed using a combination of wheat and barley malt and only enkuro as an adjunct (Table 11). Cereal combination significantly affects TSS. Beverages prepared from mixture of cereals have a higher TSS than those prepared from a single cereal (Karki and Ganga, 2011).

Table 11. Interaction effect of malt and adjunct combination on TSS

Malt combination	Adjunct combination	TSS
Wheat + barley + maize	<i>Enkuro</i>	3.71 ^d
	<i>Enkuro + yetella kita</i>	3.66 ^d
	<i>Enkuro + yetella kita + asharo</i>	4.56 ^{ab}
Wheat + barley + maize + finger millet	<i>Enkuro</i>	3.7 ^d
	<i>Enkuro + yetella kita</i>	3.9 ^{cd}
	<i>Enkuro + yetella kita + asharo</i>	4.58 ^a
Wheat + barley	<i>Enkuro</i>	3.56 ^d
	<i>Enkuro + yetella kita</i>	3.84 ^{cd}
	<i>Enkuro + yetella kita + asharo</i>	4.18 ^{bc}
SE		0.08
P-value		0.025
CV(%)		6.3

Mean values followed by the same letter are not significantly different at $P < 0.05$

Wheat + barley + maize (33% w/w) Wheat + barley + maize + millet (25% w/w) Wheat + barley (50% w/w)

Enkuro (100% w/w) *Enkuro + Yetella kita* (50% w/w) *Enkuro + Yetella kita + Asharo* (33.3% w/w)

4.3. Effect of Pasteurization on Physicochemical Properties of *Tella* (Phase Three)

4.3.1. Alcohol

Pasteurization significantly affected the alcohol content ($P < 0.05$). There was a significant difference in alcohol content among unpasteurized and pasteurized samples except for day 1, 60 and 90 (Appendix Table 3). As indicated in Table 12, on day one there was no significant difference between unpasteurized and pasteurized samples. Since it's the first day there wasn't a change in the alcohol content. On day ten, unpasteurized samples had the highest alcohol content of 1.7% and samples pasteurized at 60°C and 65°C had the same alcohol content which of 1.5%. On day 30, alcohol content of unpasteurized samples increased to 2.3% but pasteurized samples at 60°C and 65°C maintained the same alcohol content as day 20 which was 1.5%.

A decrease in alcohol content was observed on day 45 for unpasteurized samples while pasteurized samples showed a slight increase. Samples pasteurized at 60°C and 65 °C had an alcohol content of 1.7% and 1.5% respectively. There was no significant difference between unpasteurized and pasteurized samples at 60°C and 65 °C on day 60. An alcohol content of

1.6%, 1.9% and 1.65% were recorded respectively. There was a drop in alcohol content of all samples on day 90. This can be attributed to the fact that longer fermentation can further deplete glucose, maltose and fermentable carbohydrates to increase alcohol content up to certain extent beyond where there is no significant change as sugars get exhausted. Compared to the control samples, pasteurized samples maintained a consistent alcohol content. During fermentation, the metabolic activities of lactic acid bacteria and yeasts lead to production of lactic acid and ethanol from the breakdown of hexoses and pentoses (Edward and Ohaegbu, 2012). This explains the increase in alcohol content and decrease in the total soluble solid for unpasteurized sample as compared to pasteurized ones (Muyanja *et al.*, 2009).

4.3.2. pH

There was a significant difference ($P < 0.05$) in pH among unpasteurized and pasteurized samples throughout the storage period (Table 12). There was a rapid drop in pH of the control samples from initial value of 4.7 ± 0.02 on the first day to 3.4 ± 0.02 on the last day (90th day) of storage. In contrast, the pH change in pasteurized samples at the two temperatures was much slower and was statistically non significant during the storage duration. The pH of unpasteurized samples was 4.76, 4.22, 3.64, 3.57, 3.5, 3.4 and 3 on day 10, 20, 30, 45, 60 and 90 respectively. During the last day of storage (day 90), unpasteurized samples had the lowest pH value of 3 while pasteurized samples at 60°C and 65 °C had a pH of 4.5 and 4.2 respectively. The rapid decrease of pH in unpasteurized samples is attributed to the presence and growth of microbes especially lactic acid bacteria which metabolize the available fermentable sugars in to lactic acid which considerably decreases the pH during fermentation (Walter *et al.*, 2005).

4.3.3. TA

TA of unpasteurized samples was significantly different ($P < 0.05$) from pasteurized samples during the storage period except for day 20, 30 and 45 (Table 12). On day one, sample pasteurized at 65°C had the highest TA of 0.27 followed by control samples 0.25 and sample pasteurized at 60°C 0.25. Unpasteurized samples had the highest TA during the storage period. The initial TA 0.25 increased to 0.69 on day 90. The increase in titratable acidity of unpasteurized samples could possibly be due to the activities of lactic acid bacteria breaking

down sugars to produce lactic acid among other secondary products (Parawira *et al.*, 2012). In contrast pasteurized samples maintained lower and a relatively slower change in TA throughout the storage period.

4.3.4. TSS

There was a significant difference ($P < 0.05$) in TSS among unpasteurized and pasteurized samples except on day 45 (Table 12). The initial TSS of unpasteurized samples 4.08 dropped to 2.8 on day 90. Samples pasteurized at 60°C and 65°C had almost a similar decreasing rate during the storage period. However, the decrease in the pasteurized samples was more gradual than unpasteurized samples. Similarly a study made by Achi (2009) on *Obiolor*, a Nigerian fermented sorghum and millet based beverage showed there was a decline in TSS of the beverage during storage. Fermentable sugars are metabolized by microbes thus the TSS drops over time.

Table 12. Effect of pasteurization on the psychochemical properties of tella during a storage period of 90 days

Storage time (days)	PU	Alcohol (%)	pH	TA	TSS (°Brix)
1	Unpasteurized	1.5 ^a	4.76±0.15 ^b	0.25±0.002 ^b	4.08±0.09 ^a
	PU 15.86 at 60°C	1.5 ^a	4.83±0.15 ^{ab}	0.22±0.002 ^{bc}	4.05±0.09 ^a
	PU 7.79 at 65°C	1.5 ^a	4.91±0.15 ^a	0.27±0.002 ^a	4.45±0.09 ^a
10	Unpasteurized	1.75 ±0.02 ^a	4.22 ±0.02 ^c	0.37 ±0.004 ^a	3.8 ±0.05 ^b
	PU 15.86 at 60°C	1.5±0.02 ^b	4.85±0.02 ^a	0.34±0.004 ^b	4.12±0.05 ^a
	PU 7.79 at 65°C	1.5±0.02 ^b	4.62±0.02 ^b	0.34±0.004 ^b	4.15±0.05 ^a
20	Unpasteurized	2 ^a	3.64 ±0.02 ^c	0.48 ±0.04 ^a	3.7 ±0.23 ^b
	PU 15.86 at 60°C	1.5 ^b	4.85±0.02 ^a	0.45 ±0.04 ^a	3.9 ±0.23 ^a
	PU 7.79 at 65°C	1.5 ^b	4.63±0.02 ^b	0.42 ±0.04 ^a	4 ±0.23 ^a
30	Unpasteurized	2.3 ^a	3.57± 0.02 ^b	0.58 ±0.22 ^a	3.55±0.06 ^c
	PU 15.86 at 60°C	1.5 ^b	4.69± 0.02 ^a	0.62 ±0.22 ^a	3.60±0.06 ^b
	PU 7.79 at 65°C	1.5 ^b	4.69± 0.02 ^a	0.65 ±0.22 ^a	3.71±0.06 ^a
45	Unpasteurized	2.1 ^a	3.5 ±0.01 ^b	0.85 ±0.24 ^a	3.1 ±0.11 ^a
	PU 15.86 at 60°C	1.7 ^b	4.6±0.01 ^a	0.35 ±0.24 ^a	3.05±0.11 ^a
	PU 7.79 at 65°C	1.5 ^c	4.6±0.01 ^a	0.31 ±0.24 ^a	3.13±0.11 ^a
60	Unpasteurized	1.6 ±0.08 ^a	3.4 ±0.01 ^c	0.86 ±0.005 ^a	2.53±0.05 ^a
	PU 15.86 at 60°C	1.9 ±0.08 ^a	4.6 ±0.01 ^a	0.35±0.005 ^b	2.9 ±0.05 ^b
	PU 7.79 at 65°C	1.65 ±0.08 ^a	4.51 ±0.01 ^b	0.28 ±0.005 ^c	3±0.05 ^b
90	Unpasteurized	1.2 ±0.14 ^a	3 ±0.02 ^c	0.69 ±0.02 ^a	2.2 ±0.04 ^c
	PU 15.86 at 60°C	1.35 ±0.14 ^a	4.5 ± 0.02 ^a	0.28±0.02 ^b	2.7±0.04 ^b
	PU 7.79 at 65°C	1.25± 0.14 ^a	4.2 ± 0.02 ^b	0.23 ±0.02 ^b	2.81±0.04 ^a
Grand mean		1.6±0.001	4.37±0.004	0.46±0.002	3.48±0.01

Mean values followed by the same letter within a column at each storage day are not significantly different at P<0.05

Where there is no SE, SE=0

4.4. Effect of pasteurization on sensory quality of tella

4.4.1. Effect of pasteurization on flavor

There was highly significant difference (P<0.01) in flavor between the control and *tella* pasteurized samples (Table 13). The control samples and *tella* pasteurized at 60°C got highest flavor score of 3 representing the hoppy flavor which is imparted by gesho. *Tella* pasteurized at 65°C scored the lowest flavor 2.27. This could be due to the loss of volatile flavor components which are temperature dependent (Olanira, 2013). Esters are important for a

beer's volatile profile and their degradation during storage has been found to be responsible for reduced flavor (vanderhaegen *et al.*, 2006).

Table 13. Effect of pasteurization flavor and taste of *tella*

Time-Temperature(°C)	Flavor	Taste
Unpasteurized (Control)	2.93 ^a	3.71 ^a
PU= 15.86 min at 60 °C	2.73 ^a	3.65 ^b
PU=7.79 min 65 °C	2.27 ^b	3.64 ^b
P-value	<.0001	0.029

Mean values followed by the same letter are not significantly different at P<0.05

Data presented is the mean value of 30 panelists (n=30)

Flavor (1=yeasty 2= fruity 3= hoppy) Taste (1=sweet 2=bland 3=sour 4=bitter)s

4.4.2. Effect of pasteurization on taste

Pasteurization had a significant effect (P<0.05) on taste of *tella* (Table 13). Unpasteurized samples approximately scored 4 which stands for bitterness characterizing the taste. There was no significant difference between samples pasteurized at 60 °C and 65 °C. One characteristics of *tella* recognized by virtually all consumers is its bitterness. This is in agreement with Fekadu *et al.* (2013) who stated that panelists provided a sensory evaluation reflection of bitterness of *tella* samples. The bitterness is a major quality for *tella* largely attributed to a group of compounds called Iso- α -acids from the hops (Lew and Hun, 2010). Since bitterness is a good quality attribute it can be concluded that the pasteurization process didn't cause a major change in taste.

4.4.3. Effect of pasteurization on aroma

As shown below in Table 14, pasteurization had significant effect on aroma of *tella* (P<0.05) (Appendix Table 8). Unpasteurized samples had the highest mean of 3.2 followed by sample pasteurized at 60 °C with a mean aroma of 3.2. Samples pasteurized at 65 °C scored the lowest mean of 3. Cao *et al* (2011) studied varying levels of pasteurization (2–14 pasteurization units) of beer and found that an increase in pasteurization units resulted in increased loss of estery volatiles which are responsible for aroma and flavor during storage.

4.4.4. Effect of pasteurization on color

Color was significantly affected by pasteurization ($P < 0.05$) (Appendix Table 8). Unpasteurized samples has the highest color score of 3.8 followed by 3.6 for samples pasteurized at 60°C whereas the samples pasteurized at 65°C had the lowest color score of 3.1. The reason why samples pasteurized at 65°C could be attributed to the effect the pasteurization temperature. Pasteurized beers tended to have a darker color compared with the unpasteurized beer. The change in color is explained by the generation of Maillard reaction compounds and degradation of polyphenols (Cao *et al*, 2011).

4.4.5. Effect of pasteurization on mouth feel

The control and samples pasteurized at 60°C had the highest mouth feel than samples pasteurized at 65 °C. This shows that, pasteurization temperature of 60 °C has unnoticeable effect on the sensory quality in contrast to that of 65 °C which has the lowest mouth feel mean (Table 14). Therefore, sensory quality of control samples and *tella* pasteurized at 60 °C were preferable than *tella* pasteurized at 65 °C. This could be due to the pasteurization temperature since oxidation processes run faster under increased temperature it affects mouth feel (Charles, 2009).

4.4.6. Effect of pasteurization on overall acceptability

Unpasteurized samples scored the highest overall acceptability mean 3.9 followed by samples pasteurized at 60°C. Ogbadu *et al*. (1997) reported overall acceptability of pasteurized burukutu, a Nigerian sorghum beer pasteurized at 60 C for 30 minutes ranked closest to unpasteurized fresh samples. Sample pasteurized at 65 °C scored the lowest mean 3.02 (Table 14).

Table 14. Effect of pasteurization on aroma, color, mouth feel and overall acceptability of *tellas*

Time-Temperature(°C)	Aroma	Color	Mouth feel	Overall acceptability
Control	3.3 ^a	3.8 ^a	3.9 ^a	3.9 ^a
PU= 15.86 min at 60°C	3.2 ^b	3.63 ^b	3.67 ^a	3.78 ^{ab}
PU=7.79 min at 65°C	3 ^c	3.17 ^c	2.67 ^b	3.02 ^c
P-value	0.034	0.006	0.003	0.021

Mean values followed by the same letter are not significantly different at P<0.05
Data presented is the mean value of 30 panelists (n=30)

4.5. Effect of pasteurization on microbial load and shelf stability of *tella*

As indicated in Figure 8, at the first day pasteurized *tella* at 60 °C and 65°C contained no lactic acid bacteria growth whereas control samples had a mean count of 0.9×10^{-5} CFU/ml. Therefore the pasteurization employed was adequate to decrease the initial microbial load of the samples. The same result has been reported in Lund *et al.* (2012) for beer. Mean microbial count of the control sample exceeded the minimum acceptable microbial load on day 30 which was 6.1×10^{-5} CFU/ml while samples pasteurized at 60 °C and 65°C had a mean count of 0.2 and 0.3 CFU/ml respectively on the 20th day. Generally the control samples had the highest CFU/ml throughout the storage period compared to the two pasteurization temperatures. Samples pasteurized at 65°C had lower microbial count than samples pasteurized at 60°C. This is supported by Osuntogun and Aboaba (2004), who studied effect of pasteurization on shelf life stability of Kunu-zaki, fermented Nigerian beverage. Their study confirmed that pasteurized samples have much less microbial load compared to unpasteurized samples, because pasteurization destroys most mesophilic organisms like lactic acid bacteria and yeasts.

All samples showed an increase during the first three storage days followed by a relatively smooth but increasing growth followed by a decline in microbial population after the 60th day towards the last storage day. However, growth of microbial population of the control samples was rapid compared to the pasteurized samples which exhibited a much slower increase. This could be the synergic effect of depleted nutrients which are vital to microbial growth and

decrease in pH. A study made by Ellis *et al.* (2005) revealed the effectiveness of pasteurization in extending shelf life by reducing microbial content which induce spoilage. Their result showed that the shelf life of *pito* (Nigerian sorghum based beverage) pasteurization (at 60°C for 15 minutes) enabled to extend its shelf life by two months. At ambient environment unpasteurized *pito* has a shelf life of only two days.

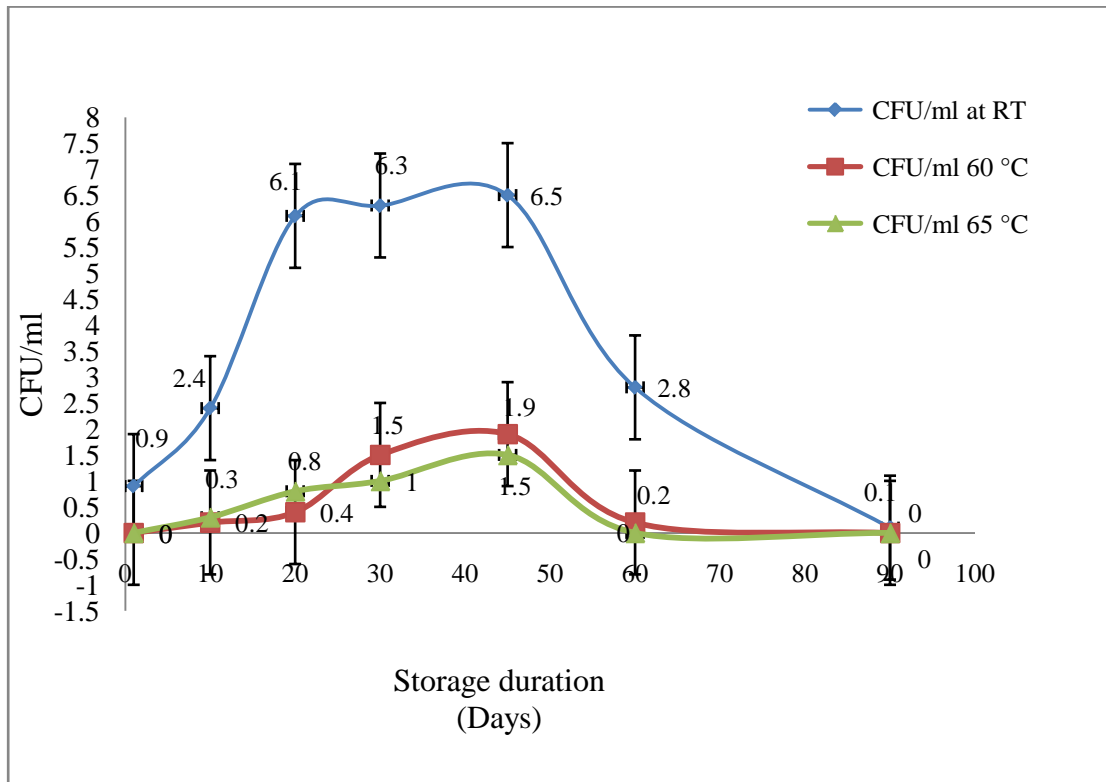


Figure 8. Mean changes in microbial population of control and pasteurized samples during storage (dilution factor 10^{-5})

5. SUMMARY AND CONCLUSION

5.1. Summary

Tella is one of the indigenous fermented beverages of Ethiopia by far the most consumed. It is produced at household and small scale level mostly by women. The role of women in the production of *tella* is a well-known fact. Twenty four percent of the respondents were widows and 13% divorced women who strive to support their children by selling *tella*. The survey revealed that less attention is given by financial institutions to this business.

Tella brewing process requires a range of ingredients which slightly differ from place to place and among brewers. The basic ingredients are local hops, malt and additional substrates which are prepared from cereals like wheat, maize, barley and finger millet. Its brewing process takes an average of six to nine days and is known to be quite laborious since it involves a series of baking and roasting.

Tella has a very limited shelf life. The survey result showed that its shelf life is limited to 3-4 days which depends on the type and quality of ingredients, processing and weather condition. Producers use different methods to extend its shelf life such as using good quality hops and malt, roasting and baking of substrates to the proper level and cooling them down to room temperature before adding, hygienic preparation and avoiding heat sources from the brewing area. Even with these efforts, its shelf life doesn't go beyond 5 or 6 days unless refrigerated.

Although its socioeconomic importance is evident, it's the least studied and documented beverage. There are very few studies which assessed the characteristics of *tella*. Hardly any efforts made by researchers to improve equipments used for processing, ingredient quality, process control, safety, product quality and storage stability. This has hinders commercializing of this indigenous beverage which can be a good business opportunity for small scale producers.

The result from laboratory studies showed that pasteurization prolonged the shelf stability of *tella* by 45 days. pH, TSS, alcohol content didn't show a significant change during this storage period. Overall acceptability of the control and *tella* pasteurized with PU of 15.86 at 60°C had no significant difference and scored the highest mean. The sensory quality of *tella*

pasteurized with PU of 7.79 at 65 °C was affected by the pasteurization temperature. The mean initial microbial count of control samples was 0.9 CFU/ml while pasteurized samples at both temperatures had a mean initial count of zero.

5.2. Conclusion

Tella serves as a sole income source for low-income women who strive to support their family. Therefore financial institutions should provide support to enable them improve and take their business to next level. Income generation by women somehow contributes to their empowerment.

Based on the findings of this study it can be concluded that pasteurization of *tella* with PU value of 15.86 minutes at 60 °C has improved shelf stability of *tella* by 45 days. Therefore pasteurization can be used to extend shelf stability of *tella*. This will help to reduce loss and improve the keeping its quality.

In view of the fact that the overall acceptability of *tella* pasteurized at 65 °C was low compared to the 60 °C, lowering the run time might reduce the effect on sensory quality. Sensory evaluation on each storage intervals would be a better approach to assess the actual changes throughout the storage period. Further studies are required to lessen the temperature effect on the sensory attributes. The fact that there was no initial microbial growth on the first day shows the effectiveness of the pasteurization process in terms of reducing the initial microbial load. Hygienic preparation and processing will reduce the initial microbial load and increase the efficiency of the pasteurization process.

Carrying out extensive researches on indigenous fermented beverages is necessary and will contribute to their improvement by transforming them in to commercial products. The results of the present study could serve as a starting point for the commercialization of *tella* which will help in developing a safer and high-quality product.

6. FUTURE LINE OF WORK

- There is scarcity of scientific literature on *tella*. More researches should be done to avoid this problem and motivate future researches.
- *Tella* is totally prepared and consumed in a traditional setting. Researches should be done to improve materials used, the processing method, final quality and safety of the beverage which can be useful inputs for the commercialization of *tella*.
- Simple and affordable small scale pasteurization equipment which can be operated by local *tella* brewers should be developed.
- Further detailed studies are needed on the effect of pasteurization temperature on the product quality, microbial profile and sensory quality of *tella*

7. REFERENCES

- Achi, O.K., 2008. Microbiology of '*obiolor*': Nigerian Fermented Non-alcoholic Beverage. *Journal of Applied Bacteriology*, 69: 321–325.
- Andrew G. H. Lea and John R. Piggot (eds), 2012 .*Fermented Beverage Production* ed 2nd edition. Springer Science and Media, New York.
- Afewerik Gebre and Bhagwan Singh Chandravanshi, 2012. Levels of essential and non-essential metals in *Rhamnus Prinoides* (Gesho) cultivated in Ethiopia. *Chemical Society of Ethiopia* 26(3), 329-342.
- Alan J. Buglass, 2011. *Hand book of alcoholic beverages: Technical analytical and nutritional aspects* .John Wiley and Sons, Ltd.
- Altay F, Karbancioglu-Guler F, Daskaya-Dikmen C and Heperkan D., 2013. A Review on traditional Turkish fermented non-alcoholic beverages: Micro biota, fermentation process and quality characteristics. *International Journal of Food Microbiology*, 167: 44-56.
- Ararso Nagari and Alemayehu Abebaw, 2013. Determination of Selected Essential and Non-essential Metal in the Stems and Leaves of *Rhamnus Prinoides* (Gesho). *Science, Technology and Arts Research Journal* 2(4):20-26.
- AOAC, 2005. Association of Official Analytical Chemists. *Official method of Analysis* 17th ed. Of AOAC International. Washington, DC, USA.
- Belay Berza and Awraris Wolde, 2014. Fermenter technology modification changes microbial and chemical parameters, improves sensory characteristics in the fermentation of tella: An Ethiopian traditional fermented alcoholic beverage. *Journal of Food Processing and Technology*, 5:4.
- Berhanu Andualem and Amare Gessesse, 2013. Isolation and identification of amylase producing yeast in Tella (Ethiopian local beer) and their amylase contribution for tella Production. *Journal of Microbiology, Biotechnology and Food Sciences* 3(1): 30-34.
- Berhanu A, 2014. Microbial profile of *tella* and the role of *Gesho* (*Rhamnus Prinoides*) as bittering and antimicrobial agent in traditional (*Tella*) beer production. *International Food Research Journal* 21(1): 357-365.
- Berhanu M., Abegaz and Teshome Kebede, 1995. Geshoidin; the bitter principle of *Rhamnus Prinoides* (Gesho). *Chemical Society of Ethiopia* 9(2): 107-114.

- Bureau of Planning and Economic Development of Oromia Regional State, 2000. Physical and Socioeconomic Profile of 180 Districts of Oromia Region. Finfinnee, Ethiopia. 248-251 pp.
- Buzrul Sencer, 2006. A suitable model of microbial survival curves for beer pasteurization. Swiss Society of Food Science and Technology, Doi: 10.1016/j.lwt.2006.10.005.
- Brandit J., Markus, 2014. Starter culture for cereal foods. *Food Microbiology*, 37:41-43.
- Briggs, D. E., Brookes P. A., Stevens R. and Boulton C. A., 2004. *Brewing: Science and Practice*. Elsevier, Amsterdam, Netherlands.
- Caballero Isabel, Carlos A. Blanco and Maria Porras, 2012. Trends in Food Science & Technology: Iso-a-acids, Bitterness and loss of beer quality during Storage. Elsevier Ltd.
- Cao, L., Zhou, G. Q., Guo, P., and Li, Y. C., (2011) Influence of pasteurizing intensity on beer flavor stability. *Journal of The Institute of Brewing*, 117(4), 587–592.
- Charles W. Bamforth (ed), 2009. *Beer: A quality perspective*. Academic Press, London, UK. pp 179.
- Clemencia Chavez-Lopez, Annalisa Serio, Carlos David Grande-Tovar, Raul Cuervo-Mulet, Johannes Delgado-Ospina and Antonello Paparella, 2014. Traditional fermented foods and beverages from a microbiological and nutritional perspective: The Colombian heritage. *Comprehensive Reviews in Food Science and Food Safety*, 13:1031-1048.
- Garafalo Cristiana, Adrea Osimani, Lucia Aquilanti, Vesna Milanovic and Francesca Clementi, 2015. Unpasteurized Commercial Boza as a Source of microbial diversity. *International Journal of Microbiology*, 194:62-70.
- Gizaw Debebe, 2006. Determination of ethanol level in beverages. An MSc Thesis presented to Department of chemistry, Addis Ababa University.
- Edward, K.C. and Ohaegbu, C.G., 2012. The effect of ginger and garlic on the microbial load and shelf life of Kunun-zaki. *Journal of Applied Pharmaceutical Science*, 02(05): 150-153.
- Ellis, W.O., Oduru I. and Terkuu, D.M., 2005. Preliminary studies on the extension of the shelf life of *pito*. *Journal of Science and Technology*, 25(1): 11-15.
- Egbere O.J., Pam K.V., Adesheyan K.D., A'kadir T., and Oyero S.K. 2009. Effect of pasteurization on survival pattern of microorganism and vitamin C retention in Kunun-zaki. *African Journal of Biotechnology*, 8(23):6603-6607.

Erasto, P., Grierson, D.S. and Afolayan, A.J. (2006). Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. *J. Ethnopharmacol.* 106(1):117-120.

Fekadu Melak, Tadele Yohannes and Khalid Siraj, 2013. Preparation and physicochemical analysis of some Ethiopian traditional alcoholic beverages. *African Journal of Food Science*, 7(11): 399-403.

Fite A., Tadesse A., Urga K, Seyoum E., 1991. Methanol, Fusel and ethanol contents of Some of Ethiopian Traditional Beverages. *SINET Ethiopia, Journal of Science* 14:1927

Gordon L. Robertson, 2009. *Food Packaging and Shelf Life: A Practical Guide*. CRC Press, FL, USA.

Hamowia A., and Saffaf, A. M., 1994. Pharmacological studies of *Vernonia amygdalina* (Del.) and *Tithonia diversifolia* (Grey). *Journal of Veterinary Medicine* 2:4-5.

Iwalokun, B.A., Efedede, B.U., Alabi-Sofunde, J.A., Oduala, T., Magbagbeola, O.A. and Akinwande, A.I. 2006. Hepatoprotective and antioxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. *Journal of Medicinal Food*, 9(4):2-4.

Kebede Abegaz, Fekadu Beyene, Thor Langsrud and Judith A. Narvhus, 2002. Indigenous processing methods and raw materials of *borde*, an Ethiopian traditional fermented beverage. *The Journal of Food Technology in Africa*, 7(2):59-64.

Kebede, T., 1994. The bitter constituent of *Gesho* (*Rhamnus prinoides*) leaves. M.Sc. Thesis presented to Department of Chemistry, Addis Ababa University.

Steinkraus K. H., (ed), 2004. *Industrialization of indigenous fermented foods*, revised and expanded edition. CRC Press.

Karki B. Dhan and Ganga P. Kharel, 2011. Effect of fermentation containers and raw materials on the chemical composition and sensory quality of fermented cereals. *Nepal Journal of Science and Technology* 12:330-339.

Khan Mohammad Zeeshan A. (2015) protection of food from microbes: A review on food preservation *International Journal of pharmacy and biomedical research* 2(1): 13-18.

Kumari Suman, Priti Guleria and Nidhi Dangi, 2015. Cereal based beverages and fermented foods: A Review. *International Journal of Enhanced Research in Science, Technology & Engineering* 4(10):134-145.

- Kubo Ryosuke, 2014. Production of indigenous alcoholic beverages in rural village of Tanzania. *Journal of The Institute of Brewing and Distilling*, 120:142-148
- Lederberge Joshua, 2000. *Encyclopedia of Microbiology*. Vol 1. Academic Press, London, UK.
- Lee Mooha, Meron Regu and Semeneh Seleshe, 2015. Uniqueness of Ethiopian traditional beverage of plant origin, *tella*. *Journal of Ethnic Foods*, 2:110-114.
- Lettish Hiralal, Balakrishna Pillay and Ademola O. O Olaniran, 2014. Stability profile of flavor-active ester compounds in Ale and lager beer during storage. *African Journal of Biotechnology*, 12(15):491-498.
- Lew Mander and Hun-Wen (eds), 2010. *Comprehensive natural products II chemistry and biology*, vol 1. Elsevier Ltd UK.
- Lyumugabe F., Gron J., Nzungie J., Bajyana E., and Thonart P., 2012. Characteristics of African traditional beers brewed with sorghum malt; a review. *Journal of Biotechnology, Agronomy, Sociology and Environmental*, 16(4):509-530.
- Lund N. Marianne, Signe Ho, Torben S. Berner and Rene Lametsch, 2012. Effect of pasteurization on the protein composition and oxidative stability of beer during Storage. *The Journal of Agricultural & Food Chemistry*, 60.
- Mogessie Ashenafi, Bekele Bahiru and Tetenike Mehari and 2001. Chemical and Nutritional Properties of 'tej', an indigenous Ethiopian honey wine: variations within and between production units. *The Journal of Food Technology in Africa*, 6(3):104-108.
- Muyanja C.M.B.K, Narvhus J.A., Treimo J. and Langsrud T., 2003. Isolation, characterization and identification of lactic acid bacteria from bushera: a Ugandan traditional fermented beverage. *International Journal of Food Microbiology*, 80:201-210.
- Muyanja C.M.B.K. and Namugumya B.S., 2009. Traditional processing, microbiological, physicochemical and sensory characteristics of Kwete, Ugandan fermented maize based beverage. *African Journal of Food Agriculture Nutrition and Development*, 9(4):1684-5374.
- Mugula J.K., S.A.M. Nnko, J.A. Narvhus and T. Sorhaug 2013. Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food. *International Journal of Food Microbiology*, 80:187-199.
- Nikita Sethi, 2016. Optimization of fermentation parameters for production of malted and alcoholic beverage from Kodo and little millet. *International Journal of Farm Science*, 6(1): 191-198.

- Ogbadu L.J., Momo-Jimosh A and Ameh J.B., 1997. Heat treatment and chemical preservatives and their effect on the keeping quality of burukutu beer: Short Communication. *World Journal of Microbiology and Biotechnology*, 13:131-132.
- Olanira A.O., Lettisha Hiralal, Balakrishna Pillay (2013). Stability profile of flavor-active ester compounds in ale and lager beer during storage. *African Journal of Biotechnology*. 12(5):491-498.
- Okafor, J. C., 1983. Horticultural promising wild plant species of the Nigerian forest zone. *Acta Horticulture*, 123:178-180.
- Osuntogun Bola and Aboaba O. O., 2004. Microbiological and physico-chemical evaluation of some non-alcoholic beverages. *Pakistan Journal of Nutrition*, 3(3): 188-192.
- Pabby Anil K., Syed S.H. Rizvi and Ana Maria Sastre Requena .2008. *Handbook of Membrane Separations: Chemical, pharmaceutical, food, and biotechnological applications*. CRC Press.
- Pandiella S. Severino, Velitchka Gotcheva, Angel Angelov, Zlatka Roshkova and Colin Webb, 2001. Monitoring the Fermentation of the traditional Bulgarian beverage Boza. *International Journal of Food Science and Technology*, 36:129-134.
- Parawira Wilson, Tusiime David and Binomugisha Sam, 2012. Microbiological changes occurring during production of traditional Rwandese Banana beer, Uragawa. *Fermentation Technology*, 1:104.
- Ray C. Ramesh and Didier Montet (eds), 2014. *Microorganism and fermentation of traditional foods*. Taylor and Francis Group, New York.
- Ryosuke Kube, 2014. Production of indigenous alcoholic beverages in a rural village of Tanzania. *Journal of Institute of Brewing & Distilling*, 12:142-148.
- Sahle A & Gashe B.,1991. The microbiology of *tella* fermentation. *SINET Ethiop Journal of Science*, 14:81-92.
- Sakamoto Kanta and Konings W. N., 2003). Beer spoilage bacteria and hop resistance. *International Journal of Food Microbiology*, 89:105-124.
- Sanni A.I., Onilude A.A. , Fadahunsi I.F., and Afolabi R.O., 1999. Microbial deterioration of traditional alcoholic beverages in Nigeria. *Food Research International*, 32:163-167.
- Shrivastava Karuna, Greeshama A.G., and Brijesh Srivastava, 2015. Improvements in traditional technology of rice and millet based fermented beverage of Arunachal Pradesh,

North East, India through scientific approach. March 18-19, Dubai International conference on chemical, environmental and biological Science.

Singh S, Kulkarni SD, Singh KK (2003) Handling banana chain-Management aspects for an Agri Industrial Approach. 6th All India Congress, National Academy of Agricultural Sciences, Bhopal.

Steinkraus K. H., (ed) 1983. Handbook of indigenous fermented Foods. Marcel Dekker, Inc. New York.

Soukand, 2015. An ethno botanical perspective on traditional fermented plant foods and beverages in Eastern Europe. <http://www.academia.edu/12876834> .Accessed online on May 4, 2016.

Tanguler Hasan, 2014. Traditional Turkish fermented cereal based products: Tarhana, boza and chickpea bread. Turkish Journal of Agriculture-food Science and Technology, 2(3): 144-149.

Vanderhaegen Bart, Hedwig Neven, Stefan Coghe, Kevin J. Verstrepen, Hubert Verachtert and Guy Derdelinckx, 2003. Evolution of chemical and sensory properties during aging of top-fermented beer. Journal of Agriculture and food chemistry 51:6782-6790

Vanderhaegen, B., Neven, H., Verachtert, H., and Derdelinckx, G., 2006). The chemistry of beer aging – a critical review. Journal of Food Chemistry, 95(3), 357–381

Walter P. Hammes, Markus J. Brandt, Kerstin L. Francis, Julia Rosenheim, Michael F.H. Seitter, Stephanie A. Voegelman, 2005. Microbial ecology of cereal fermentations. Trends in Food Science and Technology, 16:4-11.

Yeraswork Admassie and Ezana Amdework, 2010. The Aräqe Dilemma: The socioeconomics of traditional distilled alcohol production, marketing, and consumption in Ethiopia. Forum for Social Science Studies, Addis Ababa. 19p.

Zlatica Kohajdova and Jolana Karovicova, 2007. Fermentation of cereals for specific purpose. Journal of Food and Nutrition Research, 46(2):51-57.

APPENDIX

APPENDIX A. ANALYSIS OF VARIANCE TABLES

Appendix Table 1. ANOVA table of pH (Phase 2)

Source	pH		
	DF	Mean square	P>F
Malt	2	0.09172099	0.1498
Substrate	2	0.4591432	0.0002
Malt*substrate	4	0.15662716	0.1682
Day	2	0.45890617	0.0002
Malt *day	4	0.27281975	0.0291
Substrate*day	4	0.0418642	0.7727
Malt*substrate*day	8	0.13494321	0.67

Appendix Table 2. ANOVA table of TSS (Phase 2)

Source	Total soluble solids		
	DF	Mean square	P>F
Malt	2	0.2646716	0.021
Substrate	2	4.76956049	<.0001
Malt*substrate	4	0.19193086	0.0258
Day	2	1.79037531	<.0001
Malt *day	4	0.33241235	0.0013
Substrate*day	4	1.34441235	<.0001
Malt*substrate*day	8	0.07561605	0.3247

Appendix Table 3. ANOVA table of TA (Phase 2)

Source	Titratable acidity		
	DF	Mean square	P>F
Malt	2	0.00231111	0.36
Substrate	2	0.0129	0.0052
Malt*substrate	4	0.00078889	0.8392
Day	2	0.00497778	0.116
Malt *day	4	0.00134444	0.6602
Substrate*day	4	0.00411111	0.1323
Malt*substrate*day	8	0.00250833	0.3586

Appendix Table 4. ANOVA table of alcohol (Phase 3)

Alcohol content				
Source				
Pasteurization				
Day	DF	Mean squares	P>F	CV (%)
1	2	0.06824441	0.152	0
10	2	0.04166667	0.0135	2.5
20	2	0.16666667	<.0001	0
30	2	0.42666667	<.0001	0
45	2	0.18666667	<.0001	0
60	2	0.05166667	0.1671	7.1
90	2	0.01166667	0.7807	1.6

Appendix Table 5. ANOVA table of pH (Phase 3)

pH				
Source				
Pasteurization				
Day	DF	Mean squares	P>F	CV (%)
1	2	0.01055	0.0163	4
10	2	0.20385	0.0005	6.3
20	2	0.83270104	0.0001	8.5
30	2	0.84001667	<.0001	6.9
45	2	0.83637917	<.0001	3.7
60	2	0.79295	<.0001	5.3
90	2	0.62600417	0.0002	9.7

Appendix Table 6. ANOVA table of TA (Phase 3)

TA				
Source				
Pasteurization				
Day	DF	Mean squares	P>F	CV (%)
1	2	0.00155	0.002	1.6
10	2	0.00055417	0.028	1.7
20	2	0.01512917	0.1426	1.4
30	2	0.00671667	0.9386	5.1
45	2	0.13487917	0.4398	5.4
60	2	0.14606667	<.0001	1.4
90	2	0.1024125	0.0018	7.3

Appendix Table 7. ANOVA Table of TSS (Phase 3)

TSS				
Source				
Pasteurization				
Day	DF	Mean squares	P>F	CV (%)
1	2	0.09926667	0.0902	3.1
10	2	0.07625	0.0395	2
20	2	0.32666667	0.2003	8.5
30	2	0.55166667	0.0033	2.5
45	2	0.035	0.4061	5.3
60	2	0.93166667	0.0011	2.5
90	2	0.12166667	0.0078	1.7

Appendix Table 8. ANOVA Table of Sensory Evaluations

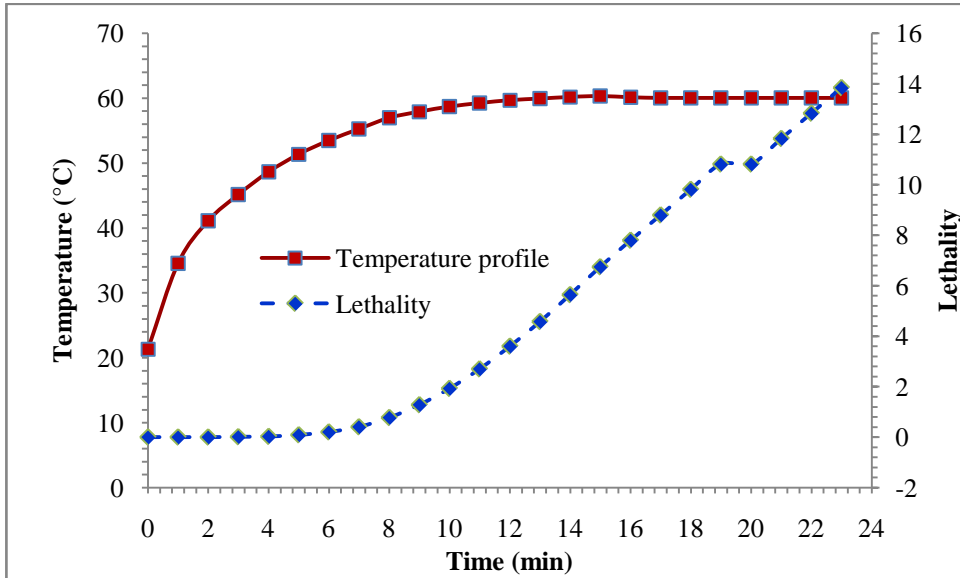
Attributes	Sensory evaluation			
	Source	DF	Mean squares	P>F
Flavor	Pasteurization	2	0.45155833	<.0001
Taste	Pasteurization	2	0.00660833	0.0297
Color	Pasteurization	2	0.50613333	0.0061
Aroma	Pasteurization	2	0.00455833	0.0341
Mouth feel	Pasteurization	2	1.81165833	0.0038
Overall acceptability	Pasteurization	2	1.035975	0.0212

Appendix Table 9. Appendix Table 9. ANOVA Table of Microbial Count

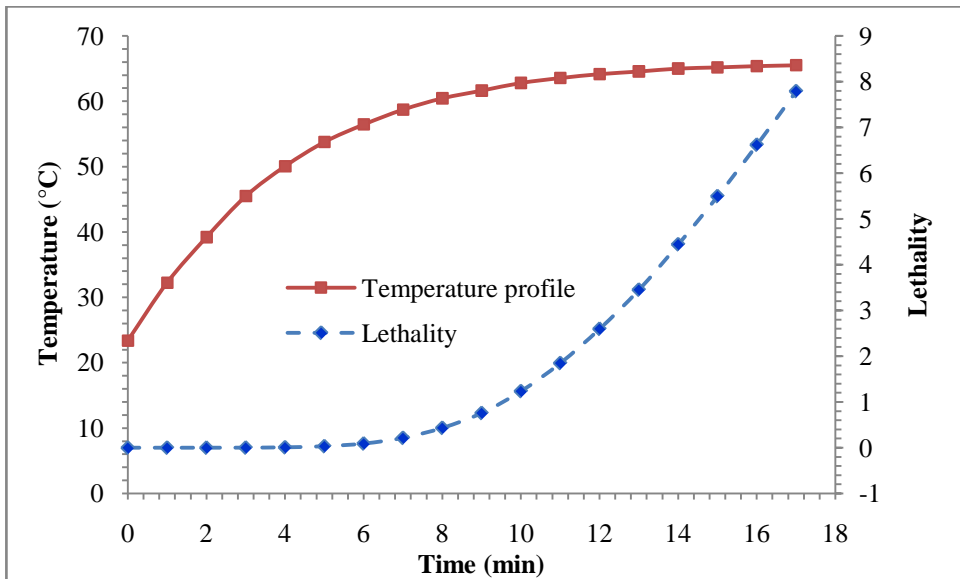
Source	Microbial count		
	DF	Mean square	P>F
Pasteurization	2	41.03166667	<.0001

APPENDIX B. TIME-TEMPERATURE PROFILE OF PASTEURIZATION

Appendix Figure 1. Time-temperature graph at 60°C



Appendix Figure 2. Time-temperature graph at 65°C



APPENDIX C. QUESTIONNAIRE USED

A questionnaire designed for the assessment of traditional tella brewing practices in Bosa Addis, Bosa Kito, Merkato Mentina, Merkato Hirmata and Becho Bore kebele's of Jimma.

Respondents' code _____

Date: _____

Zone: _____

Kebele: _____

Start of Interview: _____

End of Interview _____

Part I. Socio demographic

1. Gender A. male B. female
2. Age A. <20 B. 21-30 C. 31-40 D. 41-45 E. >50
3. Marital status A. single B. married C. Divorced D. widowed
4. Educational level A. Illiterate B. Primary education(1-4) C. Secondary cycle(5-8) D. Secondary education(9-12) E. TVET trained F. Others
5. Religion A. Christian B. Muslim C. Others(specify)
6. What is the household size? (number of family members in your house)
A. 2 B. 3-6 C. 7-10 D. > 10
7. How many children you have? A. 0. B. 1-3 C. 4-6 4. 7-9

Part II. Preparation of *tella*

8. From whom did you learn making *tella*?
A. Mother B. Sister C. Brother D. Friends E. Neighbors F. Employers G. Others(specify)
9. For how long have you been in the business?
A. <2 years B. 3-6 years C. 7-10 years D. >10 years
10. Which traditional alcoholic beverage you prepare/sell?
A. Tella B. tej C. areque D. all E. A & B F. A & C G. B & C

11. Do you prepare *tella* or you purchase and sell?
A. Yes, I prepare B. no, I purchase & sell
12. If you prepare, do you prepare by yourself or do you have workers?
A. Yes, I prepare by myself B. no, I have workers C. we work together
13. Are your families (children) involved in the preparation of *tella*? A. Yes B. No
14. In what frequency do you make *tella*? A. Every week B. Every 2 weeks
B. Every 3 weeks D. Every month E. Once a while
15. How many batches of *tella* do you prepare per week?
A. 1 batch B. 2 batches C. 3 batches D. 4 batches E. >4 bathes
16. How much litters of *tella* do you make per batch?
A. 5-10 L B.11-20L C. 21-30L D.
17. What are the lists of ingredients you use to make *tella*? _____
18. From where do you purchase ingredients?_____
19. What type of container do you use?
A. Clay pot B. plastic containers C. metal containers D. all E. others
20. How long it takes you to prepare *tella* from collection and preparation of ingredients to first day drink?
A. 5days B. 6days C. 7days D. 8days E. 9days F. 10 days G. >10 days (specify)
21. What type of malt do you use? A. wheat B. barley C. sorghum D. millet E. mix(specify type of mix & proportion)
22. Which malt mix do you use? A. wheat & barley B. wheat & millet C. wheat & maize
D. barley &millet E. barley & maize F. millet & maize G. wheat, barley& maize H. wheat, barley, maize & millet
23. Do you prepare malt by yourself or purchase it? A. yes, I prepare B. no, I purchase it
24. If your answer is yes for question 23, how do you prepare malt?
25. What do you use to prepare *yetela kita*?
A. Maize B. barley C. Sorghum D. millet E. maize & barley F. maize &millet G. maize &sorghum H. sorghum & millet I. barley, millet, maize & sorghum J. yetela kita not used
26. If you use mixes for preparing *yetelakita*, in what proportion?
27. Do you use *enikuro* or *asharo*? A. enikuro B. asharo C. both

28. If yes, what type of *injera* (fresh or dried) do you add and why?
29. Is there any different ingredient (spice) you use to make your *tella* test unique?
A. Yes B. no
30. If yes, what do you use? If you are interested to tell?
31. What type of container do you use during fermentation? A. 1day B. 2days C. 3 days
C. 3 days D. 4days E. >4days
32. When do you add yetelakita to the *Tinsis*? A. 3rd day B. 4th day C. 5th days D. 6th day
E. after 6th day(specify) F. yetelakita not used
33. When do you add *Asharo/Enikuro*? A. 3rd day B. 4th day C. 5th day D. 6th day E. 7th
day F. after 7th day(specify)
34. For how long do you allow the crude (Difdif) to ferment? A. 1day B. 2days C. 3days
D.4days E. >4days (specify)
35. What type of material do you use to close the fermentation container?
36. How many times do you open your container during fermentation time?
A. I don't open it B. one time C. two times D. three times E. more than three times
37. If your answer for question 4, is yes, why do you open it?
38. Is there any difference on the rate of fermentation during hot and cold season?
A. Yes B. no
39. If yes, in which season does fermentation takes longer? A. hot season B. cold season
40. Which season you prefer to make *tella*? Why?
41. What do you do when fermentation process is late? Why?
42. What is the amount of water you use to prepare the crude?
43. How often do you mix after dilution of the crude with water?
A. Not at all B. once C. twice D. more than twice(specify)
44. What is the water to crude ratio to make dilution?
45. What type of storage material do you use to keep *tella*?(final liquor)
A. Plastic container B. clay pot C. metal container D. all E. others
46. How many days it takes to get a clarified brew?
A. 1 day B. 2 days C. 3 days D. > 3days (specify)
47. What source of water do you use for *tella* preparation?
A. Tap B. wale C. rive D. spring E. tap &wale F. others (specify)

48. What kind of serving utensil do you use when you *sell* your *tella*?
A. Plastic B. stainless steel C. tin cans D. glass E. tin cans & glass F. others(specify)
49. For how long your fresh *tella* can be kept without showing significant change on taste?
A. 1day B. 2days C. 3 days D. 4days E. 5days F. more than 5 days
50. Has your *tella* ever been spoiled/gone bad? A. yes B. no
51. Do you have any clue why it is spoiled?
52. How do you tell if *tella* has gone bad or spoiled?(what are the signs)
53. What do you do to extend its shelf life with good quality if your *tella* is not sold on time?
54. What do you do with the spent of *tella*?
55. Do you know how many liters of *tella* you sell per day? If yes, how many litters? A. 5-11 L B. 11-15 L C.16-20 L D.> 20 L
56. How much a litter of *tella* costs?
57. Which source of energy do you use?
A. Firewood B. coffee husk C. saw dust D. cow dung

Part III. Problems

58. What are the major problems are you facing in making and selling *tella*?

APPENDIX D. SENSORY EVALUATION SHEET USED FOR THE EVALUATION OF TELLA SAMPLES

Date: _____

Time: _____

Sensory Analysis

This sensory evaluation sheet has two pages. The first page has a table with two sensory parameters; flavor and taste. Taste the samples and tick your preferred sensory aspects under each of the parameters.

The second page has a table with four sensory parameters; color, aroma, mouth feel and overall acceptability. Rate your evaluation in accordance with the following scale.

Scale:

1=Dislike Extremely

2=Dislike Moderately

3=Neither like nor dislike

4=Like Moderately

5= Like Extremely

You are provided with a total of 12 samples each with a three digit code. Please assess the samples in the given order. Please read the foot notes carefully.

N.B Please take a bite of crackers and then clean your mouth with water before you proceed from one sample to the next.

Table 1. Flavor and taste

S.No	Sample code	Flavor			Taste			
		yeasty	Fruity	Hoppy	Sweet	Bland	Sour	Bitter
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

Table 2. Color, Aroma, mouth feel and overall acceptability

S. No	Sample code	Color	Aroma	Mouth feel	Overall acceptability
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

Foot notes

- **Flavor aspects**
 - **Hoppy** –having a taste of hops
 - **Yeasty** – result of over fermentation
 - **Fruity** –having a test of fruits
- **Taste**
 - **Sweat-** related to the basic taste sensation induced by sugar likely to happen due to under fermentation
 - **Bland-** lacking in taste
 - **Sour-** an astringent(sharp) taste due over fermentation
 - **Bitter** – due to generous addition of hops
- **Aroma** is perceived by noses. To identify the aroma take short sniffs or long deep sniffs.
- **Moth feel** isthe saturation with carbon dioxide in a mouth perceived using receptors of pain

Thank you for your participation!

APPENDIX E. BEER SENSORY EVALUATION

Attribute	Scores
bitter	
sour	
sweet	
Malty	
Hoppy	
fruity	
Burnt	
Body	
Alcoholic/solvent	
Number of panelists	

Source: Journal of The Institute of Brewing, 107(5):2001