ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

ISOLATION AND CHARACTERIZATION OF THE DOMINANT YEAST IN THE TRADITIONAL BEVERAGES OF ETHIOPIA; *TELLA* AND *TEJ*

HAIMANOT ABEBE SAFAYE

A Thesis Submitted to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Applied Microbiology in Biology Department

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ABSTRACT

To date the source and type of the dominant yeast in *tej* are not known. In this study yeasts were isolated from *gesho* powder and honey. Both the ingredients contained the dominant yeast, *S.cerevisiae*, of *tej* at its consumption stage. However, *gesho* contained 10^2 to 10^3 cfu/g while honey contained 0 to 10^2 cfu/g. Thus, *gesho* was considered the major source of the dominant yeast in *tej*.

The source and type of the dominant yeast in *tella* are not known also. Yeasts were isolated from *gesho* and *bikil* powder. *S.cerevisiae*, the dominant yeast at consumption stage of *tella* was found in both the ingredients. *Bikil* contained 10^3 to 10^5 cfu/g yet *gesho* contained 10^2 to 10^3 cfu/g. Thus, *bikil* was considered the major source of the dominant yeast in *tella*.

In this study, the source and type of the dominant yeast in the beverages at consumption stage was identified. The findings of this study help to define and standardize the fermentation of the traditional beverages of Ethiopia, *tella* and *tej*.

Key words: *tella, tej, bikil, gesho,* honey

I. INTRODUCTION

In nearly all areas of the world, some type of alcoholic beverage native to its region is prepared and consumed. In Africa, fermented alcoholic beverages are consumed in different occasions such as marriage, naming and rain making ceremonies (Zvauya *et al.,* 1997), at festivals and social gatherings, at burial ceremonies and settling disputes (Steinkraus, 1983). They are also used as medicines for fever and other ailments by adding barks or stems of certain plants (Okafor, 1972). Fermented beverages produced from cereals usually referred to as beers while those produced from fruits are classified as wines (Pederson, 1979).

Indigenous fermented alcoholic beverages from different parts of the world are described (Steinkraus, 1983). Among these, information on the microbiology and biochemical properties of varieties of the indigenous African fermented alcoholic beverages is available. These include Egyptian *bouza*, Tanzanian *wanzuki*, *gongo, tembo-mnazi* and *gara*, Nigerian *palm-wine*, Kenyan *muratna* and *uragela*, and South African *kaffir* beer (Bahiru *et al.*, 2001). Indigenous Ethiopian fermented beverages include *tej* (Vogel and Gobezie 1983; Fite *et al.*, 1991; Bahiru, 2000; Bahiru *et al.,* 2001), *tella* (Sahle and Gashe, 1991; Negussie, 2008), *borde* and *shamita* (Ashenafi and Mehari, 1995; Bacha *et al.*, 1998), *areki,* traditional liquor (Desta, 1977; Fite *et al.*, 1991).

Alcohol in traditional beverages serves as source of calories valuable to the calorie-deficient villager. Ethanol has gross calorific values of 30 MJ/Kg (James and Lord, 1992). The primitive beverages provide not only calories but also B vitamins and proteins due to residues of the substrates, the fermenting yeasts and other microorganisms (Steinkraus, 1983; Bahiru, 2000). Riboflavin and nicotinic acid are found in *kaffir* beer due to synthesis of vitamins during malting and fermentation (Platt and Webb, 1964). Mexican *pulque* is

consumed as a nutritional supplement because of its vitamin content and protein. Consumption *pulque* three times a day by under-school-age children provides 2.2 to 12.4% of their calories (Sanchez-Marroquin *et al.*, 1977; Herera *et al.*, 1977). The yeasts synthesize B vitamins in Kenyan *uragua* and the alcohol provides calories (Harkishor, 1977).

Traditional recipes are handed down through generation and are still used for food processing in many developing countries (Kebede *et al.*, 2002). The traditionally fermented beverages are low-cost product in all aspect as they are usually manufactured using only rudimentary equipment. Because of their cheapness, low-income groups mostly consume them. Thus their handling and consumption often takes place under conditions of poor hygiene (Steinkraus, 1983).

In Ethiopia villagers prepare a wide range of traditional fermented foods and beverages from different raw materials such as cereals, *ensete* (false banana), honey, milk, etc. Some of the known Ethiopian traditional fermented foods are *injera, ambushes, kocho, bulla, ergo,* and *siljo.* These products, if properly exploited, could be of significant economic importance for the country (Kebede *et al.*, 2002). Most of the customs and rituals involving the Ethiopian traditional fermented foods and beverages are still prevailing today in urban areas, village communities and rural households.

Fermentation of *tej* and *tella* like other traditionally fermented alcoholic beverages relies on the microorganisms present in the substrates, fermentation vats, or equipments. So, with the variable microflora of such spontaneous fermentation variability of the product is imminent. In this study, the source of dominant yeast in *tej* and *tella* at the time the beverages were ready for consumption and characterization of the dominant yeast were studied. The findings of this study may indicate the need to isolate and characterize the

dominant yeast for subsequent modernization or industrialization, and serve as a basis for further studies.

II. LITERATURE REVIEW

2.1. Ethiopian traditional alcoholic beverages

Ethiopia is one of the countries where a wide variety of traditional fermented beverages are prepared and consumed. The various traditional fermented beverages consumed in Ethiopia consists of high alcoholic beers such as *tella;* low alcoholic beers such as *keribo, buqri, shameta, borde*, wine such as *tej* made from honey and distilled spirits such as *katikala* or *areki* (Ashenafi, 2000).

2.1.1. *Tella*

Tella is one of the Ethiopian traditional beverages, which is prepared from different ingredients. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia. It is assumed that over two million hectoliters of *tella* to be brewed annually in households and drinking houses in Addis Ababa alone (Sahle and Gashe, 1991).

Depending on the type of cereal ingredients used to make, *tella* has different names: *Amhara tella*, *Oromo tella*, and *Gurage tella* (Fite *et al.*, 1991). Amhara *tella* has *Gesho* (*Rhamnus prinoides*) and concentrated. Gurage *tella* is delicately aromatized with a variety of spices. Oromo *tella* has no *Gesho* (*Rhamnus prinoides*), and it is thick and sweet (Vogel and Gobezie, 1977).

Tella is made from different cereals. *Teff* and corn are the most popular, but in some areas barely, millet or sorghum can be used (Selinus, 1971). The way of preparing *tella* 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 *Weyra (Olea europaea subsp. Cuspidate)* for about ten minutes, in order to get it as clean as possible. Germinated grain of barley, or corn, or wheat *(bikil*), bought in the local market or prepared at home, are dried and milled. For making *bikil*, the grains are moistened in water and the moist grains are placed between fresh leaves, left to germinate for 3 days and after that dried. *Gesho* (*Rhamnus prinoides*), local hops, is available dried in the local market. The leaves of *gesho* (*Rhamnus prinoides*) is dried again in the sun for about ½ hour and after that pounded.

The leaves are separated from the stems, which need a longer time to dry. The ground *gesho* (*Rhamnus prinoides*) leaves are placed in a clay container with water and left to ferment for 2-3 days. *Gesho* (*Rhamnus prinoides*) is responsible for the bitter taste of *tella* and the bitterness of *tella* is directly related to the amount of *gesho* added during brewing. It is also thought to be the source of various chemicals (Sahle and Gashe, 1991; Kebede, 1994). It is assumed that *gesho* (*Rhamnus prinoides*) maintains acidic pH during *tella* fermentation so as to modify the nature of the mash and inhibits the growth of undesirable microorganism (Kebede, 1994).

Some of the grains intended for *tella* preparation are toasted and milled, and then mixed with water and baked on the *mitad* to prepare what is known as *kita* (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*. This mixture *enkuro*, the rest of the germinated grains (*bikil*), some *gesho* (*Rhamnus prinoides*), and water are added to the container. The mixture is kept covered overnight, after which more water is added and the

container is kept sealed for 5-7 days, until when the beverage is ready. *Tella* can be kept for 10-12 days. High quality (concentrated) *tella* is made with a relatively small quantity of water (Debebe, 2006).

According to Sahle and Gashe (1991), who made a detailed study of *tella* fermentation, there are several recipes for making *tella* and it appears as if every housewife has her own version of the recipe. The fermenting organisms of *tella* are composed of *S. cerevisiae* and *Lactobacillus* species. Increase in ethanol content (2.2 to 5% (v/v)) is directly associated with increase in the population of yeasts and decrease in reducing sugar and total carbohydrate. The pH of *tella* is in the range of 4.5 to 4.8 (Debebe, 2006).

For *tella* considered to be a good quality, the final ethanol content is in the range of 2-8% (v/v) and pH is 4-5 (Sahle and Gashe, 1991). 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 (Sahle and Gashe, 1991). According to the report, the fermentation process of *tella* is divided into four phases. The first occurs in the original mixtures of ingredients, and the second and third phases occur after successive additions of more carbohydrate materials. 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.

The report of Sahle and Gashe, (1991), also indicates that the extent of heat treatment the *asharo* (roasted barley) receives and the degree of steaming the *enkuro* (roasted barley steamed after grinding) is subjected to have the direct bearing on the color of *tella*, which is determined by the housewife preparing

the *tella. Tella* is actually a beverage of variable viscosity and having a variety of colors (ranging from grayish-white to dark brown).

Several samples of *tella* and other traditional alcoholic beverages collected from three regions of Ethiopia (Gojam, North Shoa, and Addis Ababa) were analyzed for their ethanol, methanol, and fusel oil contents by Fite *et al.* (1991). The mean values for methanol, fusel oil, and ethanol were found to be 35 ppm, 66 ppm, and 3.6%, respectively.

Filter *tella* is another beverage, which is made in the same way as the regular *tella*, but it is more concentrated. The *tella* is then filtered through a cotton cloth. After being prepared it is kept in a closed container. This type of *tella* is reported to have higher alcohol content. As reported by Desta, (1977), the average alcohol content of filter *tella* is 11.47% (v/v), with the range of 8.91 – 14.52% (v/v).

Kirari is a drink made, when the 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.

2.1.2 Tej

Tej is a home-processed, but also commercially available honey wine. It is a beverage mainly used for great feasts, such as weddings and the breaking of fasting. It is prepared from honey, water and leaves of *Gesho* (*Rhamnus prinoides*). Sometimes, widely for commercial purposes, mixture of honey and sugar could be used for its preparation. In cases where sugar is used as part of the substrate, natural food coloring is added so that the beverage attains a yellow color similar to that made from honey (Fite *et al.*, 1991). Some also add different concoctions such as barks or roots of some plants or secrete herbal

ingredients to improve flavor or potency and to attract customers. Due to concoction, adulteration practices and possibly some other reasons, producers usually are not willing to tell about additives used and their composition (Bahiru, 2000).

According to Vogel and Gobezie, (1983), during the preparation of *tej*, the fermentation pot is seasoned by smoking over smoldering *Rhamnus prinoides* stems and olive wood. One part of honey mixed with 2 to 5% (v/v) parts of water is placed in the pot, covered with a cloth for 2 to 3 days to ferment after which wax and top scum is removed. Some portion of the must is boiled with washed and peeled *Rhamnus prinoides* and put back to the fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15-20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *Rhamnus prinoides*.

Good quality *tej* is yellow, sweet, effervescent and cloudy due to the content of yeasts. The flavor of *tej* depends upon the part of the country where the bees have collected the nectar and the climate (Vogel and Gobezie, 1983).

The range for alcohol content of *tej* is very wide and values lower than 5% could be obtained in cases where the fermentation is far from complete. The higher titratable acidity combined with significant amount of alcohol would result in sweet-sour alcoholic flavor which would make *tej* preferable by consumers. This combination would also give *tej* the required microbiological stability, which would permit certain conservation without the use for highly specific techniques as observed in wine. Producers do not determine the end-point of the fermentation and *tej* is consumed while in the state of active fermentation. Aerobic conditions would result in the formation of acetic acid from alcohol making the product sourer, a condition termed as 'dryness' by consumers (Bahiru *et al.*, 2001).

Fermentation of *tej*, like other traditionally fermented alcoholic beverages, relies on the microorganisms present in the substrates, fermentation vats or equipment. As these fermentations are natural and, thus, uncontrolled, alcohol and fusel oils produced during the fermentation can be hazardous to health if produced beyond acceptable levels. With the variable microflora of such spontaneous fermentation, variability of the product is inevitable (Bahru *et al.*, 2001). The ethanol content of *tej* is reported to range from 13.2% to 13.7% (Desta, 1977), 7% to 14% (Vogel & Gobezie, 1983), 6.2% (V/V) with significant variations among samples (Fite *et al.*, 1991), and 9.07%, again with significant variations (Bahiru *et al.*, 2001). According Bahiru *et al.*, (2001), the fusel oil content of *tej* samples varies between 0.1 g/l00L and 88 g/100L. In contrast to this, Debebe, (2006) reported that neither *tej* nor *tella* has methanol and other volatile compounds.

According to Bahru *et al.*, (2001), *tej* has fewer carbohydrates than European honey wine and more proteins than grape wine. The research concludes that yeasts of the species *S. cerevisiae* are the dominant yeasts that make up 25% of the yeasts which ferment *tej*, while three other yeasts do 40% of the work between them.

The *Encyclopaedia Aethiopica*, 2009, in the entry on "Drinks," Jon G. Abbink writes that *tella* (homebrew beer) and *tej* are known under various names, differing according to language or ethno-region. *Tej* is the typical Ethiopian honey-wine or mead. In popular bars it was, and often still is, served in long-neck *berelle*, old perfume bottles, a custom dating from the late 19th century. The best *tej* is considered to be the filtered kind. *Tej* or *daadhii* (in Oromo) has become the most popular drink of many Ethiopians, not only in towns but also in countryside bars. In parts of southern Ethiopia there are similar indigenous types of fermented honey-wine, e.g. *boke*."

The book's "Honey" entry also discusses *tej*. "Honey is used in both food and drink," writes Gianni Dore, 2009. "After pressing it is used for the preparation of mead and, in its regional varieties, Tigrigna *mes*, Oromiffa *daadhii*, Amharic *tägg*, is undertaken by men or women according to social context, area and time. The regulation of the proportion of honey to water, the dosage of aromatic herbs (*gesho*, lat. *Rhamnus prinoides*; *taddwo*, lat. *Rhamnus staddo*) and the preparation and fermentation time define its alcoholic, gustative and social values, differentiating between good and domestic *mes* and commercial *mes*, from white to red to black to a bitter one, *din*, the cheapest and most unpleasant. A typical *tägg* can have around 14% alcohol."

Another section of the "Honey" entry discusses the role of honey wine in the social structure of Ethiopia: "Mead, often reserved for the chiefs, as sumptuary consumption, has symbolized a line of social demarcation: its presence in the retinues of the chiefs in war and in the shared feasting at the banquets of the élite symbolized the rank of the host and his table-companions, giving rise also to specialized roles," such as the *asallafi*, or servant who serves *tej*, and the *mälkäññ*a, the "official charged with brewing, storing and serving" the *tej*. Dore concludes: "Nevertheless, *tägg* has lost some of its status due to its association with *tägg bets*, mead houses frequented by the poor.

A 2007 report, by Beyene Tadesse and David Phillips estimates that 70% of honey produced in Ethiopia goes to the making of *tej*. Presently, most of the honey harvested goes through *tej* brewery channel. Yellow honey is usually preferred for *tej* and *berz,* a non-fermented honey solution. Although economically not so significant, *tej* is informally exported through country visitors and transitory (Harry, 2008).

2.2. Cleansing agents in *tella* and *tej* preparation

Grawa, (*Vernonia amygdalina* Del.) and *weira, (europaea* L. cuspidate (Wall. Ex Dc) Ciferri) are used for cleaning fermentation containers before the start of *tella* fermentation. *Grawa* leaves used for washing fermentation containers and *weira* splinters used for smoking fermentation jars.

2.2.1. *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. Different parts of the plant are consumed as a raw vegetable after abating bitterness by boiling and soaking in several water changes (Okafor, 1983 and Abosi and Raroseroka, 2003). It is also used as animals' feed in most parts of Africa (Onwuka *et al.*, 1989). In some parts of Africa *grawa* is regarded as a medicinal plant in Africa to treat fever, kidney problems and stomach discomfort (Hamowia and Saffaf, 1994). Additional studies also showed bactericidal effect (Erasto *et al.*, 2006), anti fungal property (Iwalokun *et al.*, 2006) and strong parasiticidal properties (Ogboli *et al.*, 2000).

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

2.2.2. *Weira* (*Olea europaea* L. subsp cuspidate (Wall. Ex DC) Ciferri)

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

of compounds that represent multichemical mechanism of defense against microbe and insect attack (Kubo *et al.*, 1985).

Weira is commonly used in Ethiopia for smoking fermentation containers of traditional beverages such as *tella*, and *tej*, containers of water, containers of milk and milk products. The smoke is reported to remove and to cause sluggish growth of coli forms in smoked milk and its products (Ashenafi, 2002). At the same time it is also observed to delay milk spoilage. *Weira* smoke give smoky flavor to water, *tella*, *tej* and milk. It has been shown that *weira* smoke contains compounds which have the ability to kill or delay the rate of growth of a number of human pathogenic bacteria and fungi (Ashenafi, 2002). However, the effect of *weira* smoke in relation to *tella* fermentation is not yet studied.

2.3. Some important ingredients of *tella* or *tej*

Some of the most important ingredients of *tella* or *tej* are dealt below.

2.3.1. *Gesho, Rhamnus prinoides* L. Herit

Gesho, Rhamnus prinoides, is a dense shrub or sometimes appears as small tree. It is spineless and evergreen. It is one of the two representative species with *Rhamnus staddo* of the family *Rhamnacaea* that are only found in Africa (Phillips, 1994). *Gesho* is commonly used for preparation of traditional alcoholic beverages, *tella* and *tej* in Ethiopia.

Rhamnus prinoides is commonly found in eastern, central and western Africa. It is found in different parts of Ethiopia, both in the wild and as a cultivated plant. It grows at elevations of 1,400 to 3,200m above sea level, in the Montana and riverine forest on the edges of clearings. The closely related species *R.staddo* A. Rich. (Vernacular name, *tsedo*) occurs only in the wild at the edges of Montana forest in wooded and scrub land in many parts of Ethiopia. *R.prinoides* is cultivated on a large scale as a field crop. It is also grown in home gardens. The leafy branches are sold fresh, or dried. (Fullas, 2003)

From a *gesho* plant leaf, stem and fruits, 20 secondary metabolites have been identified (Abegaz *et al.*, 1999; Batterham *et al.*, 1996, Dwivedi *et al.*, 1998, Helen and Juliet, 1987; and Yen *et al.*, 1998). The identified metabolites include nine anthracene derivatives, eight flavonoids and three naphthalenic compounds (Abegaz and Kebede, 1995; Abegaz *et al.*, 1999; Abegaz and Ngadjui, 1999 and Nindi *et al.*, 1999). All the metabolites are already identified from other plants except one metabolite *geshoidin* unique to *gesho* plant. It is identified as a compound with bittering character and assumed to give bitter test to the alcoholic beverages *tella* and *tej* (Abegaz and Kebede, 1995). The other metabolites from *gesho* are identified as antimicrobial agents (Paneitz and Westendorf, 1999) as pharmaceutical agents (Kartsova and Ganzaha, 2006).

2.3.2. *Bikil*

Bikil is prepared from malted cereals which may be wheat or barley. The cereal is steeped in water for about two days. The second day the water is drained off and the cereal is left packed in a container for one day to allow germination. The germinated grain is transferred in to a wide tray to allow the germinated grain to lock with each other. Then grain is smoked, sun dried and grounded to coarse powder (Nigusie, 2008).

Germination of cereal grains is initiated by water and its successful completion is signaled by the emergence of the developing roots and shoots. Following the uptake of water, hormonal signals, probably released from the embryo are believed to result in the synthesis of hydrolytic and other enzymes by the aleurone cells and scutellum (Egwim and Oloyede, 2006). Malted cereal grains have combination of enzymes, α-amylase and β-amylase, dextrinase, protease, phosphatase, peptidase and others enzymes commonly called in combination malt diastase. Malt diastase is derived from scutellum, aleuron layers and the starchy endosperm of germinated seed to hydrolyze starch molecules into simple sugar to nourish the growing seedling (Egwim and Oloyede, 2006;

Johns and Chen, 1976 and Geoffrey and Fincher, 1989). During germination and early seedling development, hydrolytic enzymes; α-amylase, β-amylase limit dextrinase and α-glucosidase enzymes convert insoluble starch granules of the endosperm into glucose (Charalampopoulos *et al.*, 2003).

Alpha amylase hydrolyzes alpha linkages in the two types of starch forms, α-amylose and amylopectin, to yield a mixture of glucose and free maltose (Barber *et al.*, 1986). Alpha amylose consists of long chains in which all the glucose units are bound in α-(1-4) linkages and the amylopectin is highly branched with back bone linkage of glycosidic α-(1-4) and the branch points are α-(1-6) glycosidic linkage.

Beta amylase attacks the non reducing ends of the two starch forms at α-(1-4) glycosidic linkage. This cleaves away successive maltose units (Barber *et al.*, 1986). However, neither α-amylase nor β-amylases can hydrolyze the α-(1-6) linkages in amylopectin branch points (Berne, 2005). This hydrolysis is brought about by limit dextrinase. Due to this reason the end product of amylase action on amylopectin is a large highly branched molecule called dextrin.

Conversion of starch enzymatically into simple sugars in malted grains is important for preparation of fermented alcoholic beverages both in large and small scales. There are yeast species that can only utilize simple sugars during fermentation process for growth and survival. They can not utilize starch as carbon source since they do not have any enzyme to hydrolyze it.

2.3.3. Honey

Honey has been highly appreciated as an alimentary product and has been largely used, since ancient times, either preventing or curing infirmity and illness or in cosmetic manufacturing. Honey is a sugary substance obtained from the nectar of the flowers or from the secretions which come from or lie on

the living parts of the plant and which honey bees' crop, transform and combine with their own specific substances and store in the honeycomb of the beehive. Honey is a product extremely rich in sugars of which glucose and fructose are outstanding. It also possesses vitamins, mineral salts and enzymes. Honey, however and despite having a high tenor in sugars and consequently a reduced water activity, an acid pH, and bactericidal substances (H_2O_2 and inhibins), it frequently suffers fermentations driven by yeasts, which make this product inappropriate for consumption. Fermentation is an irreversible phenomenon that can run in honey mainly during storage, causing significant economical losses. In such a case, honey presents a characteristic odor, increasing acid flavor and gas bubbles (Carvalho *et al.*, 2005).

The microbes of concern in honey are primarily yeasts and spore-forming bacteria. Total plate counts from honey samples can vary from zero to tens of thousands per gram for no apparent reason. Most samples of honey contain detectable levels of yeasts. Although yeast counts in many honey samples are below 100 colony forming units per gram (*cfu*/*g*), yeasts can grow in honey to very high numbers. Standard industry practices control yeast growth. Bacterial spores, particularly those in the *Bacillus* genus, are regularly found in honey (Snowdon and Cliver, 1996).

2.4. Fermentation products of beverages other than ethanol

According to some studies carried out in some African countries there is considerable evidence that home produced alcohol drinks are known to have toxic components (Fite *et al.*, 1991). A report from Zambia, as quoted by Fite *et al.*, (1991), indicates that moulds such as *Mucor* could frequently be found on the fermenting source of pectinase, the enzyme that breaks down pectinto release methanol. Methanol and fusel oil were shown to be the common contaminants of traditional alcoholic beverages in the studies of Fite *et al.*, 1991.

According to Conor *et al.*, (1979), as cited in Fite *et al.*, (1991), methanol is highly toxic and can cause blindness, insanity and even death, depending on the amount consumed. Toxic effects are usually associated with a methanol concentration in blood greater than 100g/ml.

Fusel oil is a collective name of isopentyl alcohol, 2-methyl-1-butanol, isobutyl alcohol, propyl alcohol, esters and aldehydes. It is toxic and has been shown to cause cancer in experimental animals (Fite *et al.*, 1991). A study on rats by Purchase, (1969), as described by Fite *et al.*, (1991), indicated that the toxicity of fusel oil is variable depending on the molecular weight of individual fusel oil components. These alcohols are responsible for the severe headache and thirst associated with hangover and also account for taste and flavor of alcoholic drinks.

Red wines are rich in chemicals. Non-nutrient amino acids such as tyramine are abundant in red wines and cheddar cheese. This favorite party combination can leave you with headache, confusion, and odd, inappropriate behavior. Tyramine acts something like a fight-and-flight hormone, increasing heart rate, blood pressure, and respiration, all with a sense of anxiety or panic (Gislason, 2010).

Chemical additives used to control the fermentation process further complicate alcoholic beverages. Allergenic sulfites are commonly used as disinfectants and bleaching agents in wine making. Sulfites such as Cambden tablets are used to stop fermentation. Methyl glycol has been deliberately added to some wines to sweeten the taste. Distilling of fermentation brews reduces the chemical load, but volatile products pass through the distiller along with alcohol. Only the water-clear alcoholic beverages, like vodka, are relatively free of the chemical mix (Gislason, 2010).

2.5. Yeasts

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

Yeasts dominate fungal diversity in the oceans (Bass, *et al.*, 2007). Most reproduce asexually by budding, although a few do so by binary fission. Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string of connected budding cells known as pseudohyphae, or false hyphae as seen in most molds (Kurtzman and Fell, 2005).Yeast size can vary greatly depending on the species, typically measuring 3–4 µm in diameter, although some yeast can reach over 40 µm (Walker, *et al.*, 2002).

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

Yeasts convert sugars (through a process known as fermentation) into alcohol and carbon dioxide. This trait is what endears yeasts to winemakers, brew

masters and bread bakers. In the making of wine and beer, the yeasts' manufacture of alcohol is desired and necessary for the final product; and carbon dioxide is what makes beer and champagne effervescent. The art of bread making needs the carbon dioxide produced by yeast in order for certain doughs to rise. To multiply and grow, all yeasts need is the right environment, which includes moisture, food (in the form of sugar or starch) and a warm, nurturing temperature (21.11°C to 29.44°C is best).

Wild yeasts' spores are constantly floating in the air and landing on uncovered foods and liquids. No one's sure when these wild spores first interacted with foods but it's known that the Egyptians used yeast as a leavening agent more than 5,000 years ago. Wine and other fermented beverages were made for millennia before that.

Today, scientists have been able to isolate and identify the various yeasts that are best for winemaking, beer making and baking. The two types commercially available are baker's yeast and brewer's yeast. Baker's yeast, as the name implies, is used as a leavener. It's categorized into three basic types-active dry yeast, compressed fresh yeast and yeast starters. Brewer's yeasts are special non-leavening yeasts used in beer making. Because it's a rich source of B vitamins, brewer's yeast is also used as a food supplement (Janse and Pretorius, 1995).

III. OBJECTIVES

General objective:

- To determine the major source of dominant yeast cells in Ethiopian traditional beverages; *tella* and *tej.*

Specific objectives:

- To isolate, characterize and identify the isolated yeast cells.
- To determine the number of yeast cells of the beverages and their ingredients.

IV. MATERIALS AND METHODS

4.1. Sampling

Samples of *tella, tej, bikil, gesho* and *honey* from different places of Addis Ababa were collected for this study.

A total of four samples of each of *tella* and *tej* were collected from four vending houses in Addis Ababa, i.e. one sample from each vending house. The vending houses are selected randomly. All the samples were collected in sterile screw-capped bottles. The samples were kept in a refrigerator at 4^{o}C right after collection until sample processing.

Samples of *bikil, gesho* and honey were collected from *Merkato*, the biggest open market in Addis Ababa. Again the selection is random. A total of four samples of each were considered in this study.

4.2. Isolation and taxonomical study of dominant yeasts from *tella, tej, bikil, gesho* and honey

Dominant yeasts were isolated on plates of an agar-solidified YPD medium (yeast extract, 10g; peptone, 20g; glucose, 20g; agar 20g; tap water 1,000ml) containing 50µg chloramphenicol/ml to inhibit the growth of bacteria. Yeasts were isolated following the method of (Pons *et al.*, 1986).

4.2.1. Isolation of the dominant yeast of *tella* and *tej*

One ml of *tella* and *tej* were mixed separately with 9ml sterile distilled water in a sterile flask followed by plate count method. One ml of the mixture was taken and serially diluted in test tubes each containing 9ml sterile distilled water.

This is followed by spread plating aliquots of 0.1ml from appropriate dilutions (10^{-1}-10^{-6}) on YPD agar plates which were prepared in the presence of antibiotics (Pons *et al.*, 1986). All the plates were incubated at 25^{o}C for 2 to 3 days. The colonies were transferred to slant YPDA cultures and preserved at 4^{o}C for further study.

4.2.2. Assessment of *bikil* as source of the dominant yeast of *tella*

One gram of *bikil* flour was mixed with 9ml sterile distilled water in a sterile flask for serial dilutions. The mixture of *bikil* and water was placed on shaker for 3hrs, filtered and centrifuged for 10min in 3000rpm. One ml of the supernatant was taken and serially diluted in test tubes each contain 9ml sterile distilled water. This is followed by spread plating aliquots of 0.1ml from appropriate dilutions (10^{-1}-10^{-4}) on YPD agar plates which were prepared in the presence of antibiotics (Pons *et al.*, 1986). All the plates were incubated at 25^{o}C for 2 to 3 days. The colonies were transferred to slant YPDA cultures and preserved at 4^{o}C for further study.

4.2.3. Assessment of *gesho* as source of the dominant yeast of *tella* or *tej*

One gram of *gesho* flour was mixed with 9ml sterile distilled water in a sterile flask for serial dilutions. The mixture of *gesho* and water was placed on shaker for 3hrs, filtered and centrifuged for 10min in 3000rpm. One ml of the supernatant was taken and serially diluted in test tubes each contain 9ml sterile distilled water. This is followed by spread plating aliquots of 0.1ml from appropriate dilutions (10^{-1}-10^{-3}) on YPD agar plates which were prepared in the presence of antibiotics (Pons *et al.*, 1986). All the plates were incubated at 25^{o}C for 2 to 3 days. The colonies were transferred to slant YPDA cultures and preserved at 4^{o}C for further study.

4.2.4. Assessment of honey as source of the dominant yeast of *tej*

One gram of honey was mixed with 9ml sterile distilled water in a sterile flask for serial dilutions. The mixture of honey and water was placed on shaker for 3hrs, filtered and centrifuged for 10min in 3000rpm. One ml of the supernatant was taken and serially diluted in test tubes each contain 9ml sterile distilled water. This is followed by spread plating aliquots of 0.1ml from appropriate dilutions (10^{-1}-10^{-2}) on YPD agar plates which were prepared in the presence of antibiotics (Pons *et al.*, 1986). All the plates were incubated at 25^{o}C for 2 to 3 days. The colonies were transferred to slant YPDA cultures and preserved at 4^{o}C for further study.

4.3. Isolation of active dry baker's yeast into pure culture

The active dry baker's yeast *Saccharomyces cerevisiae* by the name saf-levure, France was bought from a super market. 0.5g of it was suspended in sterile distilled water aseptically. Serial dilutions (10^{-1}-10^{-6}) were made to reduce the number of yeast cells as described above. Aliquots of 0.1ml of the suspensions were spread plated on YPDA. The cultures on the agar plate were incubated at 25^{o}C for 48hrs. The colonies were transferred to slant YPDA cultures and preserved at 4^{o}C for further study.

4.4. Characterization of yeasts

The chief characteristics used to identify and classify yeasts were (i) the microscopic appearance of the cells, (ii) their mode of sexual reproduction, and (iii) certain physiological (especially nutritional) activities (Barnett *et al.*, 2000).

4.4.1. Microscopic appearance of yeasts

Yeast cells were observed microscopically to examine their size and shape, how they reproduce vegetatively (by multilateral, bipolar or unipolar budding, by fission, by forming filaments), and the form, structure and mode of formation of ascospores.

4.4.1.1. Microscopic examination for vegetative cells

Yeast from a young, growing culture (2 days old) was inoculated into 30ml of sterile YPD broth in a 100ml conical flask. The culture was examined microscopically after incubation for 2 days at 25^{o}C (Barnett *et al.*, 2000; Lodder, 1971).

4.4.1.2. Microscopical examination for filamentous growth

Filamentous growth was detected by using the method of Barnett *et al.* (2000). Slide cultures were made as follows. A piece of filter paper, a U-shaped glass rod support, two clean microscope slides and clean cover slips, all sterilized, were put into each sterile Petri dish. Working aseptically, corn meal agar was melted and poured into a boiling-tube, wide and deep enough to hold a microscope slide. Each slide was dipped into the agar, drained a little, and replaced on its glass rod support in the Petri dish.

The medium was lightly inoculated along the length of each slide with a straight wire (the inoculums from an actively growing culture) and a cover slip placed over a part of inoculated agar. The filter paper was wetted with sterile water to prevent drying. The cultures were incubated at 25^{o}C and examined microscopically every two days for up to about two weeks.

4.4.1.3. Microscopical examination for ascospores

Since all the yeast isolates did not sporulate on one type of medium, formation of ascospores was carried out on McClary's Acetate agar and Prave's Acetate broth (Vander Walt, 1971 and Prave *et al.*, 1987).

McClary's Acetate agar (Van der Walt, 1971) is composed of 10g Glucose, 1.2g NaCl, 9.8g Potassium acetate, 0.7g Magnesium sulphate hepta hydrated, 2.5g Yeast extract, 20g agar, 1000ml distilled water, pH not adjusted.

For production of ascospores from samples' yeasts the method of Prave *et al.*, (1987) was also followed. Accordingly two types of media were prepared, i.e. presporulation and sporulation media.

The presporulation media, i.e. 20g of glucose, 2g of ammonium sulfate ($(NH_4)_2SO_4$), 2g of potassium dihydrogen phosphate (KH_2PO_4), 5g of yeast extract and 1000ml of distilled water, were kept in sterile state for 7 days. The media were inoculated with a young culture, actively growing for 2 days at 25^oC, and incubated on shaker for 3 days.

The sporulation media, i.e. 1g of glucose, 8.2g of potassium acetate, 2.5g of yeast extract, 1.86g of magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), 1000ml of distilled water, were inoculated with a drop of yeasts from the presporulation media. In contrast to the presporulation medium, this medium was used immediately after sterilization. The inoculated media were incubated at 25^oC for 3 days and examined microscopically.

Yeast samples were wet-mounted on a glass slide to observe types of ascospores. They were also heat fixed and spore stained according to Lodder (1971). Accordingly the heat fixed samples were flooded with 5% aqueous malachite green for 30-60 seconds. They were heated to steaming 3 to 4 times. The excess stain was run off under running tap water for half a minute. The preparations were then counterstained with 0.5% safranin red for about 30

seconds. The excess stain was gently washed with running tap water for half a minute. The preparations were observed both under high power (40 xs) and oil immersion objectives (100 xs). The ascospores were also observed by staining with lacto phenol cotton blue with high power (40 xs).

4.4.2. Physiological characteristics

The physiological characteristics of the dominant yeasts in the beverages were done according to Barnett *et al.*, 2000.

4.4.2.1. Fermentation of carbohydrates

The fermentation of carbohydrates was detected by using the method of Barnett *et al.* (2000). The ability to use 9 kinds of carbohydrates anaerobically, i.e. glucose, sucrose, maltose, lactose, raffinose, trehalose, starch, dextrin and xylose was assessed by looking for the formation of gas (CO_2). Large Durham tubes were used: test tubes of about 150mm by 12mm with insert tubes of about 50mm by 6mm. The tubes were filled with 10ml of YPD broth (yeast extract, 10g; peptone, 20g; glucose, 20g; tap water 1,000ml). Each tube was inoculated with a drop of a fresh yeast suspension of YPD broth, taken from an actively growing culture. The tubes were incubated at 25^{o}C for about 1 week, each day they were shaken, to help sediment the yeast, and examined for bubbles of gas in the inserted tubes (Barnett *et al.*, 2000).

4.4.2.2. Assimilation of carbohydrates

Assimilation of carbohydrates was determined according to the method of Barnett *et al.* (2000) by the auxonographic technique on nitrogen base agar medium prepared from 5g peptone, 3g yeast extract, 15g agar, 1000ml distilled water. A volume of 15ml aliquots dispensed in test tubes were sterilized, cooled to 45^{o}C and inoculated with 500µl of young yeast suspension, prepared in liquid medium. For each tube, the inoculums were well mixed with the medium and the contents were poured immediately into a Petri dish. The Petri dishes

containing the inoculated medium are left to set, undisturbed and horizontal, for about 30 min. The sugars used for the assimilation study were glucose, sucrose, maltose, lactose, raffinose, trehalose, starch, dextrin and xylose. About 5mg of the compounds were placed on the dried agar surfaces at the edge of the Petri dishes, two placed opposite each other and the third opposite a negative control area where there was no added substrate. The dishes were incubated and examined for growth every 2 days for up to about a week.

V. RESULTS

5.1. Characterization and identification of the isolated yeasts

For the identification of the yeast isolates microscopic observation and physiological tests were done.

5.1.1. Microscopic observations of the isolated yeasts

All the dominant yeasts isolated from the beverages and the ingredients did have round or oval shape and reproduced asexually by budding. They reproduced sexually by forming round ascospores in which their asci contained one up to four ascospores. All the isolated yeasts also formed pseudohyphae when they were inoculated on corn meal agar, nitrogen deficient media.

5.1.2. Physiological test of the isolated yeasts

Physiological properties of the dominant yeast isolates of the ingredients and the beverages at the time of consumption are shown in Table1. All the yeast isolates were fermentative. All of them were found to be positive for the fermentation of glucose, sucrose, maltose, and raffinose; negative for lactose, trehalose, starch, dextrin and xylose. They assimilate glucose, sucrose, maltose, trehalose and raffinose; while lactose, starch, dextrin and xylose were not assimilated by them.

The results of the microscopical observations and the physiological tests showed that the dominant yeasts isolated from the beverages and the ingredients were all *S. cervisiae*. As fermentation progresses, wild yeasts die due to their sensitivity to ethanol concentration of 2 to 6 % (v/v) while *S.cerevisiae* proliferates and completes the fermentation.

Table 1. Physiological properties of the yeast isolates (V = variable, + = positive, - = negative)

Source of yeast isolates	Fermentation									Assimilation								
	Glucose	Sucrose	Maltose	Lactose	Raffinos	Trehalo	Starch	Dextrin	Xylose	Glucose	Sucrose	Maltose	Lactose	Raffinos	Trehalo	Starch	Dextrin	Xylose
Bikil	+	+	+	-	+	-	-	-	-	+	+	+	-	+	v	-	-	-
Gesho	+	+	v	-	+	-	-	-	-	+	+	+	-	+	+	-	-	-
Honey	+	+	+	-	+	-	-	-	-	+	+	+	-	+	+	-	-	-
Tella	+	+	+	-	+	-	-	-	-	+	+	+	-	+	v	-	-	-
Tej	+	+	v	-	+	-	-	-	-	+	+	+	-	+	+	-	-	-
Baker's yeast	+	+	+	-	+	-	-	-	-	+	+	+	-	+	+	-	-	-

5.2. Mesophilic dominant yeast count of *tella* and *tej*

In this study the dominant yeasts of the beverages and their ingredients were enumerated. As shown in Table 2 and 3 the beverages contained appreciable number of the dominant yeasts.

The dominant yeast, *S. cerevisiae*, counts ranged between 10^6 to 10^7 cfu/ml in *tella* samples while 10^6 cfu/ml in *tej* samples (Table 2 and 3). There was no significant variation (CV<10%) in yeast counts between samples from the same source in both beverages. Differences in yeast counts among samples collected from different sources was not significant (CV<10%) either in both beverages.

Table 2. Counts (log cfu/ml) of yeast cells from *tella* samples obtained from different sources.

Vending house	Min	Max	Mean	SD	%CV
A	7.18	7.33	7.24	0.08	1.10
B	6.85	7.09	6.98	0.12	1.72
C	6.56	6.78	6.64	0.12	1.81
D	6.30	6.48	6.39	0.09	1.41
All houses			6.81	0.16	2.35

Table 3. Counts (log cfu/ml) of yeast cells from *tej* samples obtained from different sources.

Vending house	Min	Max	Mean	SD	%CV
A	6.48	6.60	6.55	0.06	0.92
B	6.70	6.83	6.78	0.07	1.03
C	6.30	6.48	6.39	0.09	1.41
D	6.56	6.78	6.64	0.12	1.81
All houses			6.59	0.35	5.31

The mean dominant yeast populations among samples of all vending houses are the same and all the vending houses have no significant differences (CV<10%) between their mean values in their yeast populations in the beverages (Table 2 and 3).

5.3. Mesophilic dominant yeast count of *bikil* flour, *gesho* leaf powder and honey

In the process of *tella* and *tej* fermentation there is no deliberate yeast introduction to starter culture. The yeast cells involved in the fermentation process must come from the *bikil*, *gesho* and/or honey. As shown in Table 4 and 5 *bikil* and *gesho* contained appreciable number of yeast cells. However, comparatively *bikil* contained more of the dominant yeast cells of the beverages. Honey may or may not contain yeasts (Table 6).

The dominant yeast, *S.cerevisiae*, counts ranged between 10^3 to 10^5 cfu/g in *bikil* samples. There was no significant variation in yeast counts between samples from the same source (CV<10%). However there were significant

differences (CV>10%) among samples of the vending houses in their dominant yeast populations (Table 4).

Table 4. Counts (log cfu/ml) of yeast cells from *bikil* samples obtained from different sources.

Shop	Min	Max	Mean	SD	%CV
A	4.75	5.57	5.15	0.41	7.96
B	3.00	3.63	3.38	0.33	9.76
C	3.48	4.00	3.73	0.26	6.97
D	4.43	5.00	4.72	0.29	6.14
All shops			4.25	0.80	18.82

The dominant yeast, *S. cerevisiae*, counts ranged between 10^2 to 10^3 cfu/g in *gesho* samples. There was no significant variation in yeast counts between samples from the same source (CV<10%). Differences in yeast counts among samples collected from different sources were not significant either. There were no significant differences (CV<10%) among samples of the vending houses in their dominant yeast populations (Table 5).

Table 5. Counts (log cfu/ml) of yeasts from *gesho* samples obtained from different sources.

Shop	Min	Max	Mean	SD	%CV
A	2.78	3.30	3.03	0.26	8.58
B	2.49	2.68	2.57	0.10	3.89
C	2.66	3.10	2.92	0.23	7.88
D	2.51	2.78	2.64	0.14	5.30
All shops			2.79	0.26	9.32

The dominant yeast, *S. cerevisiae*, counts ranged between 0 to 10^2 cfu/g in honey samples. There was no significant variation in yeast counts between samples from the same source (CV<10%). There were significant differences (CV>10%) among samples of the vending houses in their dominant yeast populations (Table 6).

Table 6. Counts (log cfu/ml) of yeast cells from honey samples obtained from different sources.

Shop	Min	Max	Mean	SD	%CV
A	2.25	2.40	2.33	0.08	3.43
B	2.30	2.46	2.39	0.08	3.34
C	2.00	2.15	2.09	0.08	3.83
D	0.00	0.00	0.00	0.00	0.00
All shops			1.70	1.04	61.18

VI. DISCUSSION

The dominant yeast isolates of the beverages and the ingredients were *S.cerevisiae*. Yeasts of the genus *Saccharomyces* were considered by Vogel Gobezie (1977) to be responsible for the conversion of sugars to ethanol in *tej*. According to Sahle and Gashe (1991) fermenting organisms in *tella* are composed of *Saccharomyces* species (mostly *S.cerevisiae*) and *Lactobacillus* species.

Tella and *tej* are prepared from different ingredients and involve a mixed culture of yeast and bacterial cells. To date the source of the dominant yeast in the beverages and their taxonomy is not known. From the results of this study, all the ingredients and the beverages did have *S. cerevisiae* as their dominant yeast. And it was found that *bikil* was the major source of the dominant yeast in *tella* while *gesho* was the major source of dominant yeast in *tej*.

The process of *tella* fermentation involves addition of *gesho, bikil,* and *yetela-kitta* (Vogel and Gobezie 1983). Except *bikil* and *gesho*, all the other ingredients (*yetela-kitta, enkuro* and the like) are heat treated before addition to the fermentation vat. Thus, to determine the source of yeast cells in *tella* in this study attention was focused on *gesho* and *bikil*. The dominant yeast, *S.cerevisiae*, counts ranged between 10^3 to 10^5 cfu/g in *bikil* samples while 10^2 to 10^3 in *gesho* samples. And *bikil* was deduced to be the major source of yeast in *tella*.

Tej fermentation, like other traditional beverages of Ethiopia, is a natural fermentation and no starter culture or other modern techniques are used (Fite, 1991). So, the fermentation depends upon the microorganisms present in the environment. *Tej* is prepared from honey, or honey and sugar, water and *gesho*. Thus, to determine the major source of the yeast cells in *tej* attention was given to honey and *gesho*. The dominant yeast, *S. cerevisiae*, counts ranged between 10^2 to 10^3 cfu/g in *gesho* samples while 0 to 10^2 in honey samples. And *gesho*

was considered the major source of the dominant yeast in *tej* because it contained greater number of the yeast than honey. Similar to findings in this study, *S. cerevisae* was considered by Bahiru, (2000) to be the dominant yeast in *tej*.

The unrecorded data of this study showed that *gesho* had both antibacterial and antifungal roles. In addition to these, Sahle and Gashe, (1991), indicated that the bitter taste of *tella* is directly related to the amount of *gesho* added during brewing just like hop. According to Sahle and Gashe, (1991), there is a decline in pH during the fermentation of *tella*. Acidic pH is a selective factor in the growth or inhibition of microorganisms. Hence, initial pH drop is considered as a desirable characteristic because of the inhibition of potential spoilage organisms. In commercial beer production, acidic pH increases the quantity of extractable from hops (Humulus lupulus) and also enhances the activities of enzymes. The acid also modifies the nature of the mash and inhibits the growth of undesirable microorganisms. Even though, studies on *gesho* have not been done, it is suspected that *gesho* might be serving similar purpose to that of hops in beer production in the acidic environment which is created during *tella* fermentation.

A few investigations like the works of Nigussie, (2008), had shown the possibility of setting up standards, using starter culture and developing a good system for creating anaerobic condition in the fermentation to ensure an extended shelf-life of the beverages. In addition to this, in this study the major source of the dominant yeast, *S.cerevisiae*, in *tella* and *tej* was found. Thus, the findings of this study and others are indispensable for subsequent modernization or industrialization, and serve as a basis for further studies.

VII. CONCLUSION AND RECOMMENDATIONS

Like other African beverages the fermentation process of *tella* and *tej* is also by uncontrolled fermentation.

Variation in the quality and stability of products are the recurring disadvantages.

In this study, the kind and the source of the dominant yeast in the beverages were identified.

This study indicated that *S. cerevisiae* is the dominant yeast in *tella* and *tej* at the time they were ready for consumption.

The dominant yeast was found in all of the ingredients (*bikil*, *gesho* and honey).

Bikil was found to be the major source of the dominant yeast in *tella* while *gesho* was the major source of the dominant yeast in *tej*.

In modernization, the cost of product should be given special attention.

Unrecorded data in this study indicated that *gesho* had antibacterial and antifungal roles. Thus, further researches need to be conducted toward the roles of gesho.

The effect of *grawa* leaves and *weira* in relation to *tella* preparation is not studied yet. Thus, further researches on the functions of *grawa* and *weira* are also needed to be done.

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DECLARATION

I, the undersigned here by, declare that this thesis is my original work, has not been presented for a degree in any other university and that sources of materials used for this thesis have been dully acknowledged.

Haimanot Abebe Safaye

Signature_____ Date_____

This thesis has been submitted for examination with my approval as the university advisor

Dr. Dawit Abate

Signature_____ Date_____