ADDIS ABABA UNIVERSITY SCHOOL OF

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VARIABILITY IN SOME MICROBIOLOGICAL AND CHEMICAL PROPERTIES OF "*TEJ*" BETWEEN AND WITHIN SOME VENDING HOUSES IN ADDIS ABABA.

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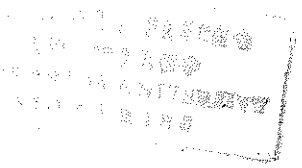
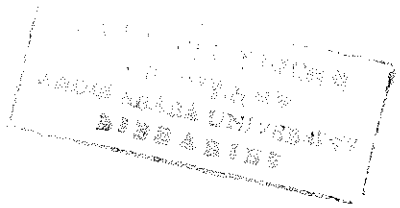
A THESIS SUBMITTEDTO THE SCHOOL OF GRADUATE STUDIES OF THE ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED MICROBIOLOGY IN BIOLOGY DEPARTMENT.



TO MY MOTHER EHITAGEGNEHU AYELE.

MAY YOU REST IN PEACE !



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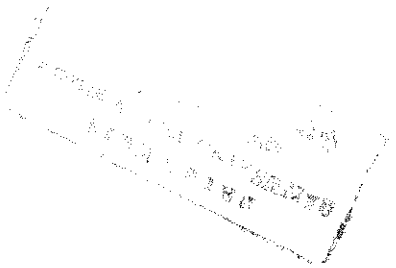

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ABSTRACT

'tej' being honey wine, is one of the primitive types of wines that are not crystal clear, but cloudy, effervescent, containing residues of substrates, the fermenting yeasts and other microorganisms. Honey wines are prepared and consumed in many parts of the world. *'tej'* is consumed widely in many parts of Ethiopia and is prepared from honey, or honey and sugar, water and *Gesho (Rhamnus prenoides)*. Roots, stems or leaves of plants are added assuming an increase in the alcohol content and its potency. Like any other spontaneous fermentation, *'tej'*, in its fermentation, relies on the microorganisms that inhabit the must from different sources. The substrates, utensils, equipment, and the environment are responsible for the randomization of the microflora. In such spontaneous fermentation and un-optimal physical and chemical conditions, variability in the product quality and stability becomes inevitable. So, investigating the variability and its extent in some microbiological and chemical properties of *'tej'* at the time it was ready for consumption, was the objective of this study.

At consumption stage, *'tej'* was highly loaded with yeasts and Lactic acid bacteria. Members of different genera of yeasts, members of the bacterial genera of *Lactobacillus, Pediococcus, Streptococcus, Leuconostoc*, were isolated and identified. Of the yeast isolates, species of *Saccharomyces* were dominant, followed by *Kluyveromyces, Debaromyces, Hansenula, Endomycopsis* and *Pichia* species. Generally, the homofermentative (52.09%) were higher in counts than the heterofermentative(47.91%)

lactic acid bacterial population.. All the isolates from the aerobic mesophilic bacterial plates were identified as members of *Bacillus*.

All the studied microbiological and chemical properties of *'tej'* had significant variability among samples within each vending house. Also, except yeast and lactic acid bacterial populations and fusel oil contents, all the other microbiological and chemical properties of *'tej'* studied had statistically significant variability (ANOVA, $p < 0.004$ for all) between the mean values of all the vending houses. Generally, all samples also showed variability in their total alcohol, total lipid, protein, and reducing sugar. Variability in fusel oil, titratable acidity and total carbohydrate was also noted. So, *'tej'* at the time it was ready for consumption had statistically significant variability both within and between vending houses in the microbiological and chemical properties.

I. INTRODUCTION

Nearly all humans, except those who do not consume alcohol for religious reasons, incorporate fermented alcoholic beverages into their diet. In many Middle East and African countries consumption of alcohol and lactic acid fermented products are common (Hesseltine, 1979; Platt and Webb,1964). These products have many advantages. They are superior in digestibility and nutritive value compared to the unfermented counterparts (Sanni, 1992; Zvauya,*et,al.*, 1997). Combination of pH reduction, lowering of redox potential, competition for essential nutrients and the production of inhibitory compounds inhibit the growth of spoilage and pathogenic microorganisms (Gilbert *et al.* 1983; Steinkraus, 1983).

Ethanol serves as a source of calories, undesirable for the overfed west but valuable to the calorie-deficient villager. Ethanol has gross calorific values of 30 MJ/kg (James *et al.* 1992). The primitive wines provide not only calories but B vitamins due to residues of the substrates, the fermenting yeasts and other microorganisms (Steinkraus, 1983).Also alcohol in traditional beverages serves as a calorie source (Steinkraus, 1983). Approximately 35% of the caloric intake of South African mining workers comes from kaffir beer (Platt and Webb, 1964). The consumption of Nigerian palm wine varies from 0.5 to 1.5 liters per person per day. Bassir(1968)stated that a liter of good oil palm wine fermented from the oil palm(*E. guineensis*), provides approximately 300 calories from its sugar and alcohol. Although alcohol provides certain amounts of calories, there are evidences that high level of alcohol intake increases the risk of buccal cavity, pharyngeal, Oesophageal, and liver cancer (McGlashan, 1969; Slaga, 1980;; Higginson and Muir, 1981).

Fermented alcoholic beverages provide certain amounts of B vitamins and proteins due to their microbial contents(Steinkraus, 1983; Escobar, 1977;Platt and Webb,1964). Riboflavin and nicotinic acid are found in two-folds in kaffir beer due to synthesis of vitamins during malting and fermentation (Platt and Webb, 1964). The major nutrients in palm wines are sugars and B complex vitamins. $VitB_{12}$ increases with time from 190 to 500 µg/liter at 24^{th} hour(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, 1977; Herera *et al.* 1977). The yeasts synthesize B vitamins in Kenyan *uragua* and the alcohol provides calories (Harkishor, 1977).

The quality of traditionally fermented alcoholic beverages is largely outside social control and surveillance, so consumption of large quantity of low-alcohol traditional beverages may expose people to potential health risk due to impurities, contaminants and additives. Excessive drinking of alcohol may potentiate the development of malnutrition. It is known that malnutrition may play a role in the development of cancer in alcoholics, who may consume 25 to 50% of their calories in the form of alcohol (Nikander *et al.* 1991). Peneda *et al.*(1994) suggested that the hepatotoxicity of ethanol in alcoholic beverages is enhanced by interaction with its congeners and acetaldehyde. They also suggest that alcoholic beverages are not equivalent in their potential to cause liver damage.

Fermented beverages produced from cereals are usually referred to as beers while those produced from fruits are classified as wines (Pederson, 1979). Fermentation of a variety of foods or blends of fruits, cereals, milk, sap, honey, molasses and/or other foods are also wines. They are designated by the substrates from which they were made. Honey has been diluted and fermented

alone for centuries. It has been used as a sweetening agent in Mexican *pulque*, *Shoshone* of Indians, *Contaruru* of Equador, Roman *Mulsum*, and in beers of ancient Egypt (Pederson, 1979). Honey was an important ingredient of the meads or metheglins prepared by the ancient Nordics.

Honey wine (mead, Metheglin) has been an indigenous fermented beverage for thousands of years. Honey wines are also made on small scale in England, France and Poland. Honey wines are primitive types of wines that are not crystal-clear products. Instead they are cloudy, effervescent containing residues of substrates and fermenting yeasts and other microorganisms (Steinkraus, 1983).

'Tej' is one of the primitive type of wine. It is prepared from honey, or honey and sugar, water and *Gesho* (*Rhamnus prenoides*). Concoction by adding barks, leaves, roots, etc. of some plants have become common practices. Addition of hops to honey wines is a very old technique (Prescot and Dunn, 1959). Originally *'tej'* was found only in the homes of the royalty and noble men (Vogel and Abeba Gobezie, 1977). During the regime of Emperor Menelik II, an increase in the drinking population and illegal kosher trade resulted alcohol-related problems such as criminal acts, traffic accidents, violation of laws, etc. Then, the Emperor proclaimed that both partners, those who buy and those who sell *'tej'* and *araki* should meet at those particular places restricted or in accordance to the law as *'Mesheta bet'* (Paulos, Gnogno, 1991).

Fermentation of *'tej'*, 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 microbiological and chemical variability (both qualitatively and quantitatively) and the extent

of the variability at the time *'tej'* was ready for consumption were studied. The findings of this study may indicate the need to define and standardize the product for subsequent modernization or industrialization, and serve as a *basis* for further studies.

II. LITERATURE REVIEW.

Ever since fermented liquor was produced, the prime interest has been its intoxicating effect (Dickes and Nicholas, 1976). In nearly all areas of the world, some type of alcoholic beverage native to its region is prepared and consumed (Banwart, 1983).Different fermented foods and beverages constitute a major portion of peoples diet in Africa.(Sanni, 1992). Lactic acid and alcoholic fermentations are widely used in Africa to ensure flavor acceptability of raw grains (Platt and Webb,1964).

Some of the fermented alcoholic beverages from different parts of the world include Egyptian *bouza* (Sanni, 1992); Tanzanian *Wanzuki, Gongo, Tembo-mnazi* and *Gara* (Nikander *et al.* 1991); Nigerian palm-wine (Maduagwu *et al.* 1979); *Tiquira* of Brazil (Nascimento *et al.*1992); Philippine *basi* (Sanchez, *1977*; Tanimura *et al.* 1978); Kenyan *muratina* and *uragua* (Harkishor, 1977); Mexican *pulque* (Gonclaves de Lima, 1977);Indian jack-fruit wine (Dahiya and Prabhu, 1977); African kaffir beer (Novellie, 1976); Honey wines of Europe and USA(Steinkraus, 1983); Ethiopian *'tej"* and *'Tella'*(Vogel and Abeba Gobezie,1977 ; Alemu Fite *et al.*1991) and Nigerian *Buruktu* and *Sekele* (Sanni, 1992; Faparusi, 1970a). More over wine and beer are prepared and drunk differently under different names in many parts of the world.

Some possible hazardous outcomes in the modernization or substitution of traditionally fermented foods and beverages have been observed in some parts of the world. Kaffir beer production is the large modern industry founded on tribal art of African origin (Novellie, 1968). It is very important in the nutrition of the Bantu tribe in South Africa (Platt, 1964; Platt and Webb, 1946). The trend of change in the substrate ratio, which is the primary factor for the loss of vitamins, was evident. (Novellie,1968).The same was the fate for the Mexican *pulque* which was loosing its calorie-

providing potential because of modernization (Sanchez-Marroquin, 1977 Herrera *et al.* 1977). Modification and modernization of traditional fermented foods and beverages should only be undertaken when the nutritional value can be maintained, correct information on traditional production is obtained, and organoleptic and aroma characteristics are maintained (Sanni, 1992).

Fermented alcoholic beverages around the world are consumed in different occasions. Fermented beverages constitute a major part of the diet of traditional African rural homes serving as inebriating drinks and weaning foods, in addition to their role in social functions such as marriage, naming, and rain making ceremonies (Zvauya *et al.* 1997). Kenyan *muratina* and *uragua* are drunk largely at festivals and social gatherings (Harkishor, 1977). Palm wines (*Toddys*) are fermented and consumed under different names in different parts of the world. Palm wine has a special place in traditional celebrations and ceremonies such as marriages, burials, and settling disputes (Ayenor and Mathews, 1972; Fapparusi, 1977; Merican, 1977; Wong and Jackson, 1977; Odeyemi, 1977). In West Africa in addition to their use as *beverages, Toddys* are also used as medicines for fever and other ailments by adding barks or stems of certain plants (Okafor, 1972b). Native gods are appeased or worshiped through the use of palm wines. Mexican *pulque* is consumed on birthdays, weddings, on picnics as an accompaniment to local foods. Indian jackfruit wine is a social and tribal beverage (Dahiya and Prabhu, 1977).Ethiopian *'tej'* and tella are consumed on weddings, holidays and other festive occasions as well.

The traditional fermented alcoholic beverages of the world could be grouped into two groups based on the types of substrates (sugars or starch or sometimes a mixture of both) used for their preparation and production of ethanol (Steinkraus, 1983). Alcoholic fermented beverages resulting from fermentation of sugar constitute the first group and are produced in a wider

hydrolysis is accomplished by amylolitic moulds and yeasts as in the Japanese *sake* (Yoshizawa,1977;), and Korean *Yakju* and *Takju* (Park *et al.* 1977).

Various groups of microorganisms are involved in the fermentation of different alcoholic beverages. The dominant microorganisms in the Philippine *basi* are *Saccharomyces, Endomycopsis* and lactic acid bacteria (Sanchez, 1977; Tanimura *et al.* 1978b). The primary ethanol producing yeast in the Mexican *pulque* is *Saccharomyces cerevisiae,* with other yeasts of less importance *(Endomycopsis, Pichia, Torulopsis* spp) (Sanchez-Marroquin and Hope, 1953).

There are many and different kinds of indigenous fermented beverages in Ethiopia, but, information on these beverages is scanty. *Tella* is one of the traditional beverages which is prepared from different substrates *Tella* is a fermented traditional beverage of variable viscosity and having color ranging from greyish-white to dark-brown. Barley, wheat, maize, millet, sorghum, teff, etc. are used for preparation of *tella*(Vogel and Abeba Gobezie, 1977; Pederson, 1979; Alemu Fite *et al.* 1991). *Tella* is a malt beverage beer. *Tella,* by far is the most commonly consumed alcoholic beverage in Ethiopia. Well over 2 million hectoliters of *tella* is thought to be brewed annually in households and drinking houses in Addis Ababa (Samuel Sahle and Berhanu Abegaz, 1991).

Depending on the type of cereal ingredients used to their locality, *tella* has different names (Alemu Fite *et al.* 1991). Amhara *tella* has *Gesho(Rhamnus prenoides)* and concentrated. Gurage *tella* is delicately aromatized with a variety of spices. Oromo *tella* has no *Gesho(Rhamnus prenoides),* it is thick and sweet (Vogel and Abeba Gobezie, 1977). There are several recipes for making *tella* and it appears as if every housewife has her own version of the recipe. Samuel Sahle

and Berhanu Abegaz (1991) made a detailed study of Tella fermentation. The fermenting organisms of tella are composed of *Saccharomyces cerevisiae* and *Lactobacillus* species. Increase in ethanol content (2.2 to 5%) is directly associated with increase in the population of yeasts and decrease in reducing sugar and total carbohydrate (Samuel Sahle and Berhanu Abegaz, 1991). The pH of '*tella* is in the range of 4.5 to 4.8. There was variability in the number and kinds of microorganisms involved during the different stages of fermentation.

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 (Alemu Fite *et al.* 1991). The mean values for methanol, fusel oil, and ethanol in '*tella*' were found to be 35ppm, 66ppm,and 3.6% respectively.

'Tej' is another traditionally fermented alcoholic beverages consumed in Ethiopia. It is home processed, but is also commercially available. For *tej* sugars are the principal fermented carbohydrates.In commercial preparations, a mixture of honey and sugar could be used in the preparation. In some cases where sugar is used as a substrate, natural food coloring is added so that the beverage attains a yellow color similar to that made from honey (Samuel Sahle and Berhanu Abegaz, 1991; Alemu Fite *et al.* 1991). As in the other alcoholic beverages, such as beers, different concoctions such as barks or roots of some plants or secrete herbal ingredients (McGlashan, 1969) are added to improve flavor or potency and to attract customers (Alemu Fite *et al.* 1991). A handful of roasted barley, also wood or bark from the shrub *Rhamnus tsado(*similar to *gesho)* is added to initiate fermentation (Vogel and Abeba Gobezie, 1977). Due to concoction, adulteration practices and possibly some other reasons, producers usually are not willing to tell about substrates used and the preparation.

High quality *'tej'* is yellow, sweet, effervescent and cloudy due to the content of yeast (Vogel and Abeba Gobezie, 1977). Tej is not a crystal-clear product as wines known in the western world, instead they are turbid, effervescent slurries containing residues of substrates, the fermenting yeasts, and other microorganisms (Steinkraus, 1983). The flavor of *'tej'* depends also up on the part of the country where the bees have collected the nectar and the climate (Vogel and Abeba Gobezie, 1977).

Crude honey makes better mead than refined honey, because the pollen serves as a yeast nutrient (Vogel and Abeba Gobezie, 1977). According to Steinkraus and Morse, 1966(as cited in Steinkraus, 1983), light yellow honey is deficient in nitrogen and growth factors needed by yeasts, so, its natural fermentation is prolonged requiring months rather than days for completion. Dark honey contains more pollen and consequently more growth factors than light honey and consequently ferments more readily.

Originally *'tej'* was found only in the homes of royalty and noblemen, and lately upon the permission from this social stratum, the preparation and consumption of *'tej'* widespread following social hierarchy from the palace up to the kosher trade or *'mesheta bet'*. According to Vogel and Abeba Gobezie (1977), during the preparation of *'tej'*, the fermentation pot is seasoned by smoking over smouldering *Gesho(Rhamnus prenoides)* 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 *Gesho (Rhamnus prenoides)* and returned back to the fermenting must. The pot is covered and fermented continuously for another 5 days if warm or for 15-20 days if cold. The mixture is stirred daily and finally filtered through cloth to remove sediment and *Gesho (Rhamnus prenoides)*.

'Tej' fermentation, like other traditional beverages of Ethiopia, is a natural fermentation and no starter culture or other modern techniques are used (Alemu Fite *et al.* 1991). So, the fermentation depends upon the microorganisms present in the environment. Yeasts of the genus *Saccharomyces* are responsible for conversion of sugars to ethanol (Vogel and Abeba Gobezie, 1977).The ethanol content of *'tej'* is reported to range from 13.2 % to 13.7% Belachew Desta(1977), 7% to 14%(Vogel and Abeba Gobezie,1977) and 6.2%(v/v) with significant variation among samples and between samples of different sources (Alemu Fite et al.,1991). The mean value of methanol and fusel oil reported by Alemu Fite et al (1991), for *'tej'* samples collected from three different regions of Ethiopia (Gojjam, North shoa, and Addis Ababa) were 47ppm and 104ppm respectively.According to Spector(1956), the LD_{50} of isopropyl alcohol given orally to rats was 5840mg/kg body weight , while the LD_{50} of isobutyl alcohol in the same route was 2460mg/kgbody weight.On the otherhand, Purchase(1969) reported that the LD_{50} of isoamyl alcohol given to female rats orally was 4.0g/kg, while the LD_{50} of n-hexyl alcohol in the same route was 0.72g/kg body weight.No data are available hitherto about changes in nutritional value, microbial ecology, principal chemical changes, and other characteristics

Fermented foods and beverages constitute a major portion of people's diet in Africa. However, preparations of these products are still traditional family arts and the fermentation is by uncontrolled inoculation (Sanni, 1992). It is, therefore, not surprising that much effort has been directed towards the study of microbiological and chemical changes occurring during fermentation of traditional African foods (Zvauya,, *et al.* 1997).

Unless fermentation conditions are optimized in order to obtain consistent products, the complex microflora implicated in spontaneous fermentations, are unpredictable and they lead to the

variability in the quality and stability of the product. Mixed-culture of spontaneous fermentation is the rule in African food fermentation (Sanni, 1993) and the disadvantage of mixed-culture and uncontrolled fermentation is the recurring variations in product quality.

The basic substrates used in the preparation of most of alcoholic beverages, particularly home-processed ones, are adequate media for the growth of many types of microorganisms (Pederson, 1979). There is little doubt, therefore that considerable variation exists among the various preparations depending upon the flora other than yeasts.

The variability in palm wines is the result of the random microbiological make up of the fermenting sap (Faparusi, 1973). The major problems in palm wine fermentation are excessive acidity, sediment and production of hydrogen sulfide which imparts a characteristics and undesirable flavor (Okafor, 1972b).

The persistence of some yeasts such as *Pichia membranaefaciens* and *Candida mycoderma*, beyond the primary fermentations of brined olives is due to their ability to grow on lactic acid produced during the primary fermentation (Ingram, 1958). *Pichia* and *Hansenuala* occur as contaminants in alcoholic liquors. They can use alcohol as a source of carbon, and are nuisance in the fermentation industry.

III. MATERIALS AND METHODS

1. Sampling.

A total of ten *''tej''* vending houses in and around central Addis Ababa were considered in this study.Most of them were located along or near main roads. *''tej''* vending houses designated as A, B, C...were located in *Arat-kilo* area D in *Amist-kilo*, E in *Addis-ketema*, F and G in *Kechene*, H in *Wube-bereha*, I and J in *Kirkos*. *''tej''* vending houses B, D, G produced and vended at cheaper prices (Birr 2 to 2.40/litre), A, C, F, I, J produced and vended products at medium prices (Birr 5 to 6/litre) and E and H vended more expensive types (Birr 8 and 12/litre respectively).

Table-1 Location, volumes and cost of samples of *'tej'*/liter

Code of vending houses	Location or name of places where samples were collected	Volumes of samples collected (in ml.)	Cost of samples Birr/liter
A	Arat kilo	500	5.00
B	Around Ras Mekonnen bridge	500	2.00
C	Arat kilo	500	5.00
D	Amist kilo	500	2.40
E	Addis ketema	1000	8.00
F	Kechene	500	6.00
G	Kechene	500	2.00
H	Wubebereha	1000	12.00
I	Kirkos	750	5.33
J	Kirkos	500	6.00

Samples of *'tej'* were collected in autoclaved screw-capped glass bottles. A total of 199 Samples of one liter were collected from the ten sampling sites. Samples were immediately brought to the laboratory for microbiological and biochemical analyses.

2. Microbiological analysis

Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysis. Microbiological analysisTwenty-five ml of *'tej'* samples were blended with 225ml of maximum recovery diluent (MRD) prepared from 0.1g peptone, 0.85g NaCl in 1000ml distilled water and autoclaved at 121^{O}C(Roberts et al.,1995).Measured volumes of appropriate dilutions were plated on the following culture media.

2.1 Aerobic mesophilic count.

A volume of 1.0ml of 10^{-1} and 10^{-2}dilutions of *'tej'* samples were pour-plated on nutrient agar (Difco) and incubated at 30 - 32°C. Colonies were counted after 48 hours of incubation.

2.2 Aerobic spore formers.

Samples were heat treated at 80°C for 10 minutes (Hayes, 1995) in a water bath (Gallenkamp) to destroy all vegetative cells and selectively plate spores of aerobic spore formers. One ml of 10^{-1} and 10^{-2} dilutions were pour-plated on Nutrient agar (Difco) and incubated at 30 - 32^{0}C for 48 hours for counting.

2.3 Coliform count.

A volume of 1.0ml of 10^{-1} and 10^{-2}dilutions of *'tej'* samples were pour-plated on Mac Conkey agar No.3.(oxoid) The plates were incubated at 30 -32^{0}C for 20 - 24 hours and checked for pink colonies.

2.4 Counts of Enterobacteriaceae.

A volume of 1.0ml of10^{-1} and 10^{-2} dilutions of *'tej'* samples were pour-plated Mac Conkey agar NO.3(oxoid) to which 10g glucose per liter was added. The plates were incubated at 30 -32^{0}C for 20 - 24 hours and checked for any pink colonies.

2.5 Counts of Lactic acid bacteria.

A total of 0.1ml of 10^{-3} and 10^{-4} dilutions of *''tej''* samples were spread plated on pre-dried surfaces of de man Rogosa Sharpe(MRS) agar and incubated under anaerobic conditions at 30 – 3 2^{O}C for 48h for counting.

2.6 Counts of yeasts and moulds.

For the enumeration of yeasts and moulds, volumes of 0.1ml of 10^{-3} and 10^{-4} dilutions of *'tej'* samples were spread plated on pre-dried surfaces of Chloramphenicol- Bromophenol-Blue agar (CBB) consisting of yeast extract (oxoid) 5.0, Glucose (BDH) 20, Chloramphenicol 0.1, Bromophenol blue (BDH) 0.01,agar (oxoid) 15 (g/liter distilled water) and pH adjusted to 6.0 to 6.4. Yeast colonies were counted after incubating at 25 - 28^{0}C for 4 to 5 days. The presence or absence of big, spreading colonies with hairy margins was checked under stereoscopic microscope. Smooth, non-hairy colonies lacking extensions at margins were counted as yeasts.

2.7 Assessment of flora

About 10 to 20 colonies with different color and morphology were randomly picked from countable plates used for aerobic mesophilic count and purified by repeated plating. Yeast isolates were maintained on Wickerham'sYeast extract-Malt extract (YM) Agar (oxoid), pH adjusted between 5 and 6.. The following morphological and confirmatory biochemical characteristics were used to differentiate the isolates.

2.7.1 Cell morphology.

Morphological characterization of the pure cultures were made by observing wet mounts, hanging drops, and gram stained preparations of young cultures under microscope. The morphological characteristics used were:

2.7.1.1. Motility

A hanging drop was prepared by placing a drop of the young test culture on a glass cover slip. The cover slip was inverted over a thin ring of plasticine(SIGMA) on a microscope slide (Roberts *et al.* 1995), observed under oil immersion of a microscope and the motility or non-motility of the organisms was recorded.

2.7.1.2. Cell shape-

Cell shape-was examined using gram stain and/or wet mounts under oil immersions and whether the cells are rods, coccobacilli, cocci (regular shapes) or branched, coryneforms, pleomorph (irregular shapes) was recorded (Singleton, 1995).

2.7.1.3. Endospores-

The presence or absence of endospore was examined by observing spore stains of 48 hr cultures (Hayes, 1995).

2.7.1.4. Cell grouping –

Wet mounts were examined and all grouping was recorded as single, pairs, chains, clusters, or tetrads (Singleton, 1995).

2.7.2 Catalase production test

The test for the production of enzyme catalase was made by placing a drop of 3% H_2O_2 solution on a glass microscope slide (Roberts *et al.* 1995) and gently rubbing a colony or two of a 24hr culture of the test organism in the H_2O_2 drop. Evolution of gas bubbles was considered as a positive reaction for the test.

2.7.3. Gram staining and/ or KOH test.

Gram staining was made for the entire isolate but for doubtful observations KOH test was made according to Gregersen (1978). In the latter test, one to two drops of 3% KOH solution was

dropped on a glass microscope slide that was placed on a dark background. A colony or a few colonies were picked from a young pure culture and stirred (5-10 stirrings) into the KOH with a wire loop. The changing of the KOH solution to viscous solution and formation of a thread of slime following the loop upon raising was considered as a positive test for gram negativity while a watery suspension, as a positive test for gram positivity.

2.8 Grouping of Lactic acid bacteria.

Isolates from MRS plates that were gram positive, catalase negative, non-sporing, non-sporing, cocci or rod shaped bacteria were considered as Lactic acid bacteria. They were grouped as homo- and heterofermenters by their ability to produce gas in 5% Glucose(BDH) in MRS broth(MERCK) containing inverted Durham's tubes and incubated at 30 to 32 ^{0}C for 4 - 5 days.

2.9 Identiufication of of yeast isolates.

Yeast isolates were characterized based on their morphological, cultural, sexual, and physiological characteristics (Lodder, 1971)

2.9.1 Morphology of vegetative cells and vegetative reproduction(asexual).

The shape of vegetative cells, the occurrence of budding and/or fission was examined recorded after 2 to 3 days of growth in 20 ml of 2% Glucose-Yeast extract- Peptone water(prepared from 20g Glucose, 10g bacto peptone, 5g bacto yeast extract in 1000 ml distilled water, pH was not adjusted(van der Walt, 1971). The nature of the budding (unipolar. Bipolar, multipolar, or unilateral) was also checked .The shape of vegetative cells (spheroidal, subglobose, ovoid, cylindrical, elongate, ogival, lunate, etc.) was also studied and recorded by wet-mounting after growing the yeast isolates on Malt Extract agar agar was used.

2.9.2. Cultural characteristics

After inoculating and incubating at 28^0C for 2 to 6 weeks in 2% Glucose-Yeast extract-Peptone water, formation of a sediment, a ring, islets or a pellicle was studied and recorded (van der Walt, 1971). The yeast growth was examined on Malt Extract agar and growth type was recorded as mucoid, butyrous, friable, coherent, or matted. The surface and margin of growth was also examined for rough and smooth or mat and glistening forms. Pigment production was also checked. Presence of filamentous growth and formation of ascospore was studied using slide culture technique(Barnette *et al.* 1979) on Potato Glucose agar.

2.9.3 Verification and staining of ascospores.

Since all the yeast isolates did not sporulate on one type of medium, formation of ascospores was carried out on the following different media:

A. McClary's Acetate agar (van der Walt, 1971) composed of 10g Glucose, 1.2g NaCl, 9.8g Potassium acetate, 0.7g Magnesium sulphate hepta hydrated, 2.5g Yeast extract, 20g agar.1L distilled water. pH not adjusted.

B. Potato Glucose agar, according to (van der Walt, 1971) was prepared as follows: 100g of potato was peeled, grated and soaked in 300ml tap water for 48 hours in a refrigerator. The mass was filtered through clothe, autoclaved for 1hr. at 15 lbs. Then 230ml of this potato extract was added to 770ml tap water containing 20g agar and 20g Glucose, dissolved by steaming, autoclaved at 121^0C and 15lbs for 15 minutes. pH not adjusted.

C. Gorodkowa agar(Barnette *et al.* 1979) was prepared by dissolving 0.1%(w/v) Glucose,0.5% (w/v) peptone, 0.5%(w/v) NaCl, 2%(w/v) agar in 1000ml distilled water,pH not adjusted. autoclaved at 121^OC for 15 minutes PH not adjusted.

D. Yeast ascospore agar (Atlas, 1993)Prepared from 30g agar, 10g potassium acetate, 1.0g Glucose, and 1000ml distilled water. ph After dissolving by steaming, the medium was sterilized by autoclaving at 121^OC and 15lbs for 15 minutes. pH not adjusted.

2.9.4. Physiological characteristics.

2.9.4.1. Fermentation of carbohydrates.

Fermentation of carbohydrates was determined in the carbohydrate fermentation basal medium of Wickerham (Van der Walt, 1971) prepared from 4.5g yeast extract(oxoid), 7.5g peptone, sufficient amount of bromothymol blue,1000ml distilled water. 2% sugar solutions (Raffinose, 4%) were prepared by dissolving in 0.5%(w/V) yeast extract (Oxoid) solution. Four ml aliquots of the nitrogen base medium dispensed in cotton plugged test tubes (150mm X 12mm). In the tubes were put inverted Durham's tubes and the sugar solution (aftere separately autoclaved), according to Van der Walt (1971). After sterilization, one ml aliquot of the sugar solutions were asceptically added to the test tubes containing the nitrogen base.The sugars used for this test were: Raffinose, Galactose, Mannose (Fluka A.G.chem.), Fructose, Glucose, Maltose, Glycerol, D-Mannitol, Sucrose, Lactose, Soluble starch, α,α -Trehalose, L-Rhamnose, Salicin(SIGMA) and the indicator Bromothymol blue(Fluka A G chem.).

A loopfull of young cultures was inoculated into the tubes and incubated at 28^{O}C for 24 to 72 hours. Negative results within this period of time were incubated for another 72 houyrs.A blank consisting of inoculated basal medium devoid of any carbon source served as control. The accumulation of gas in the Durham's tubes and change of color of the indicator was recorded as positive result.

2.9.4.2 Assimilation of carbohydrates.

Assimilation of carbohydrates was determined according to the method of Barnette *et al.*(1979) by the auxanographic technique on nitrogen base agar medium prepared from 5g Caesin peptone(oxoid), 3g yeast extract(oxoid), 15g washed agar, 1000ml distilled water, pH was

adjusted to 6.0 to 6.4. A volume of 15-18 ml aliquots dispensed in test tube were sterilized as 121^{O}C for 15 minutes, cooled to 45^{O}C and inoculated with young yeast cultures. These were thoroughly mixed and poured into sterile petridishes to solidify. The sugars and alcohol used for the assimilation study were Mannose, Raffinose, Lactose, α,α-Trehalose, Soluble starch, Maltose, Fructose, Sucrose, Glucose, Galactose, L-Rhamnose, Salicin, D-Mannitol, and Glycerol. About 5mg of the compounds were placed on the dried agar surfaces at the edge of the petridishes opposite to one another. Three test compounds and Glucose were tested on each petridish. The petridishes were incubated at 28^{O}C for upto about 10 days and examined for growth under illuminated colony counter.

2.9.4.3 Assessing growth at high concentration of Glucose

Growth at high concentration of Glucose was studied on slants of Yeast extract-glucose agar according to Barnette et al (1979). The medium consisted of 0.5%(w/v) yeast extract, 2%(w/v) agar, in 1000ml distilled water and 500g and 600g glucose were separately added to the media, dissolved by steaming, and dispensed in 5-6ml in test tubes. The test tubes were autoclaved at 121^{O}C for 15 minutes. Slants were prepared and lightly streaked with active young cultures and incubated at 28^{O}C for up to a week.

2.9.4.4 Detecting production of extra-cellular starch-like (amyloid) compounds.

The production of amyloid compounds was studied according to the method of Barnette et al(1979). Lugol's solution was prepared consisting of 5g iodine and 10g Potassium iodide(both from BDH) in a 100ml distilled water. This solution was diluted to 1:5 for use with sterile distilled water. One drop of this solution was shaken with YM broth (pH 3.7) in which the yeast isolates were grown at 28^{O}C and pH 3.7 for 48 hours. The development of Blue, purple, or green was recorded as a positive result.

3. Biochemical analyses.

3.1 pH values of samples

The pH of samples was measured by dipping the electrode of a digital pH meter(Tuv Goprütte Sicharheit) in the samples.

3.2 Titratable acidity as lactic acid.

The titratable acidity of samples was determined in triplicates according to Zvauya *et al.* (1997). Five ml of sample was transferee into a 250-ml conical flask and diluted with distilled water. This solution was titrated to a faint pink color against 0.1M NaOH(Aldrich) using 3 drops of 1% phenolphthalein as an indicator. The amount of acid was calculated as percent lactic acid according to the formula.

$$\% \text{ lactic acid} = \frac{\text{ml of 0.1MNaOH X Normality of NaOH X MWt. Of acid}}{\text{ml of sample X 10}}$$

And then the amount (in grams) of lactic acid/100ml of samples was calculated from the total amount of titratable acidity value obtained.

3.3 Total alcohol content.

The total alcohol content of samples was determined by specific gravity method of AOAC 19. 004 (Williams, 1984). A 100ml volumetric flask was filled to the mark with sample, transferred to a distillation flask to which a condenser was fitted. The volumetric flask was repeatedly rinsed with distilled water, and the water was added to the flask. The content of the flask was distilled slowly at a uniform rate from 30 to 60 minutes using longer time for higher percentage of alcohol. Then, about 95 ml of distillate was collected in a 100-ml volumetric flask, the flask filled to the mark with distilled water and mixed by inversion. The specific gravity of this distillate was determined at 20^0C.

3.4 Fusel oil content.

The fusel oil contents of samples were determined by spectrophotometric method of AOAC. 9.084 (Williams, 1984). A solution of 1g *p*-dimethyl aminobenzaldehyde (DMAB) in 5 ml H_2SO_4 and 90 ml distilled water was diluted to 100 ml in a volumetric flask with distilled water. According to Ikeda et al(1956), such colorimetric method based upon the Komarowsky reaction, i.e., the reaction of isobutyl and isoamyl alcohols with aromatic aldehydes in conc. H_2SO_4 solution is, satisfactory in determining fusel oil in alcoholic beverages. The method has advantages of speed, accuracy, high sensitivity of color complexes and less sensitivity to minor variations in procedure (Webb and Ingraham, 1963). A stock solution of standard fuseloil solution was prepared by weighing 2g isobutyl alcohol and 8g isoamyl alcohol) in two one-liter-volumetric-flasks) and diluted to one litre, one with water and the other with ethanol. This standard solution contained 10g/100L fusel oil. Working standards were prepared by diluting 0.1 - 8.0ml aliquots of the aqueous standard solution to 100ml with alcoholic solution of proof expected for diluted samples when pipetted into analysis tubes.

During the distillation of samples, 100ml of sample with 20ml of water was added into a distillation flask (Pyrex) fitted to a condenser and distilled according to AOAC method 9.124 (Williams, 1984), slowly but steadily collecting volume of distillate equal to that of sample.

Two ml aliquots of distilled samples were diluted ten-fold and 2ml aliquot of the dilution, 2ml of distilled water (for reagent blank),and 2ml aliquots of standards were pipetted into test tubes. These were placed in an ice bath for 3 min. One ml of the coloring reagent *p*–Di Methyl Amino Benzaldehyde (DMAB) was added into each tube, shaken and replaced into the ice bath for 3min. With tubes still in the ice bath, 10ml of chilled Conc. H_2SO_4 was added down the side of tubes.

Tubes were shaken and replaced in the ice bath for 3 min. The tubes were transferred to boiling water bath and boiled for 20 min.Then transferred to ice bath for 3-5 min., and to room temperature bath .The % transmittance of the developed color of samples and standards was read on spectronic 1001 spectrophotometer (Milton Roy) at 540nm using a 1cm light path cuvette. A standard curve was developed by plotting gram fuseloil/100L against %T (%transmittance). After developing the standard curve, the %T of samples were converted to gram fuseloil/100L from the standard curve. The amount of fuseloil/100L in the original sample was obtained by multiplying the g fuseloil of samples read from the standard curve by dilution factor. Two levels of standards were run each time of determination with each series of samples.

3. 5. Protein content.

The amount of total available protein was determined according to Schacterl and Pollack(1973) using alkaline copper reagent and Follin's Ciocalteus phenol reagent.

A working solution of follin's Ciocalteus phenol reagent(SIGMA) was prepared by taking 0.5ml of a 1N and adding 4.0ml distilled water.

Albumin standard solution was prepared by disolving100mg of crystalline bovine serum albumin fraction V (SIGMA) in distilled water and kept refrigerated. Working standards were prepared daily by diluting this stock solution.

In the assay 1ml of the alkaline copper reagent was mixed with 1ml of ten-fold diluted samples in a test tube ,then the mixture was allowed to stand undisturbed for 10 minutes. Fourml of the phenol working reagent was added forcibly and rapidly by using 5ml pipette fitted to a filler. The test tube were placed in a water bath at 55°C for 5 minutes, cooled rapidly in a beaker of tap

water for one minute. The absorbance of the developed color was read at 650 nm by using spectronic1001 spectrophotometer (Milton Roy) through a 1 cm light path cuvette.

3.6 Total carbohydrate as glucose.

The amount of total carbohydrates in samples was determined colorimetrically by the Phenol-sulphuric acid method (Dubois, 1951; Dubois *et al.* 1956). A 5% solution (v/v) of phenol was prepared by dissolving 5g phenol(SIGMA) in a100ml in glass dstilled water. A 95.5% (v/v) H_2SO_4 was prepared by mixing 95.5ml conc. H_2SO_4(SIGMA) with 4.5ml distilled water.

A ten-fold dilution of samples were prepared and 2ml of these dilutions were transferred in to test tubes. 1ml of the 5% phenol solution was mixed with the samples using vortex mixer(Lab-Line instruments, inc.) . Then, 5ml of the 95.5% H_2SO_4 was added rapidly directing its stream against the liquid surface with a 5ml pipette fitted to a filler (Gallenkamp) and mixed with the vortex. The tubes were allowed to stand for 10-20 minutes in a water bath at 25-30^{0}C.

The standard stock solution of glucose was prepared by dissolving 100ml of glucose in 100ml distilled water. Serial dilutions of 0.05, to 0.6mg/ml of the standards were prepared and run through the procedure above. All solutions were prepared in duplicates. The absorbance of the yellow color was read at 490 nm using spectronic 1001 spectrophotometer (Milton Roy) through a 1cm light path cuvette. A standard curve was developed by plotting mg glucose in the standard solution/ml of samples as abscissa against absorbance as ordinate on a graph paper. The absorbencies of sample solutions were converted to mg glucose/ml sample from the standard curve.

3.7 Reducing sugar content as glucose.

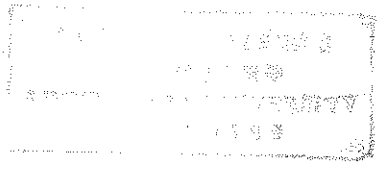
The reducing sugar content of samples was determined colorimetrically by the Nelson- Somogy method (Nelson, 1944; Somogy, 1951). Copper reagent A was prepared by dissolving 25g of anhydrous sodium carbonate, 25g of rochelle salt, 20g of sodium bicarbonate, and 200g of sodium sulphate (anhydrous) in 800ml distilled water. This solution was diluted to 1liter in a 1000ml volumetric flask (pyrex) and filtered. Copper reagent B was prepared as 15% $CuSO_4$. $5H_2O$ (in a distilled water) containing 2 drops of H_2SO_4 (conc.) per 100ml.(all chemicals from SIGMA) Reagent C (Arsenomolybdate reagent) was prepared by dissolving 25g of ammonium molybdate in a 450ml distilled water.Twenty-one ml conc. H_2SO_4 was added and mixed. To this solution was added separately prepared solution of 3g sodium arsenate (hepta hydrated) dissolved in a 25ml of distilled water. Reagent C was mixed and placed in an incubator (Gallenkamp) at 37^0C for 48 hours. Reagent D (the working reagent) was prepared by mixing 25 parts of reagent A with one part of reagent B.

A ten-fold diluted one ml of sample was mixed with 1ml of reagent D in a test tube with a vortex mixer (Lab-Line instruments inc.). This mixture was immersed in a boiling water for 20 minutes, cooled immediately under running water and allowed to stand for 5 minutes at room temperature. One ml of reagent C was added and mixed until the cuprous oxide dissolved. The final volume was adjusted to 25ml with distilled water. The absorbance of the blue color was read at 500 hm using spectronic 1001 spectrophotometer (Milton Roy) through a 1cm light path cuvette. Standard serial glucose solution containing 0.05, 0.1...0.6mg glucose/ml distilled water was prepared. From the absorbance of the standard solutions, a standard curve was developed by plotting mg/ml of glucose in standard solutions on the X-axis against absorbance on the Y-axis. The absorbance of the samples was converted to mg of glucose/ml sample from the standard curve developed.

3.8 Total lipid content.

The total lipid content of samples was determined gravimetrically by Bligh and Dyer method (Jayaraman, 1992; James, 1995; Holmes and Peck, 1993). A mixture of chloroform and methanol (2:1 v/v) was used for extraction. A volume of 50ml sample was transferred into a 250ml conical flask.Thirty ml of the chloroform-methanol mixture was poured into it while vigorously shaking the flask. For complete extraction the flask was stoppered and kept in a dark place at room temperature for overnight. At the end of this period 20ml chloroform and 20ml of water containing NaCl was added to remove the non-lipid material. To separate the phases, the resulting solution was centrifuged at 2000g using angle-head centrifuge (Gallenkamp).Centrifugation resulted three layers. The upper colored aqueous layer of methanol with all water-soluble materials was discarded. A thick pasty interface was separated from the clear lower layer of chloroform containing all the lipids by filtering through glass wool. The chloroform layer was taken into a pre-weighed vial and evaporated by using Rota evaporator (Buchi RE 121) in-vacuo at 45-50°C. The weight difference was recorded as the weight of total lipid content of samples.

For the identification of isolates, monograph of Lodder (1971),keys of Barnett et al(1979),for standard values of specific gravity of ethanol standard tables from James(1995),and Williams (1984)were used. In the statistical analyses of the numerical data, average values and 95% confidence limits were calculated by the appropriate statistical methods as described by Steel and Torrie(1960).The statistical significance of variability between the mean values of vending houses were checked as described by Conover(1980) and James(1995) with the appropriate statistical package(SPSS inc. 1990-1995 Microsoft corp.).



IV. RESULTS

1. Microbiological observations

Aerobic mesophilic bacterial counts were generally low in all samples obtained from the various *'tej'* samples (data not given). In vending houses G and I, 6 and 7 of the samples, respectively contained no detectable amounts of aerobic mesophilic bacteria. Vending houses B, E, F and J also had less than four samples with undetectable number of aerobic mesophilic bacteria. Although most samples with detectable number of aerobic mesophilic bacteria contained $<10^3$ cfu/ml, vending houses D, F, G, H, and J had a considerable number of samples with counts ranging between 10^4 - 10^5 cfu/ml. Six of the ten vending houses had between 2 and 7 samples, which did not contain detectable number of aerobic spore formers. In cases where they were detected the number was usually $<10^3$ cfu/ml. It can be stated that the various *'tej'* samples contain markedly low counts of aerobic mesophilic bacteria and aerobic spore formers. No *Coliforms* and members of *Enterobacteriaceae* in all samples obtained from all sources.

According to the spss outputs (table6), the mean aerobic Mesophilic Bacteria (AMB) and aerobic spore formers' counts of all the vending houses were different from each other (ANOVA, $\rho <$ 0.001, for both) with higher chi-square values of 35.47 and 38.06 respectively.

The lactic acid bacteria (LAB) were the most dominant of the microorganisms in the various samples collected from all *'tej'* samples. Counts ranged between 10^5 and 10^7 cfu/ml (Table 2). No significant variation was noted in counts between samples within a vending house (CV < 10%) or samples among vending houses.

Of the lactic acid bacteria, *Lactobacillus* spp were among the frequenty encountered groups. Samples from each vending house had comparable numbers of heterofermentative and homofermentative *Lactobacillus* spp. However, the heterofermentative had relatively higher counts of heterofermenters in all cases except vending houses A, B and C (Table 3). In general no significant variation was noted in counts of homofermenters within all the vending houses (CV <10%) except in vending house G. Significant variations were, however, noted in counts of the heterofermenters among samples within vending houses in B, F and I (CV >10%). There was no significant variation in mean counts of both heterofermenters and homofermenters among vending houses.

Table 2 Counts (log cfu/ml) of lactic acid bacteria in various *'tej'* samples obtained from different vending houses.

Vending house	Min	Max	Mean	SD	%CV
A	6.12	7.12	6.83	0.27	3.9
B	6.03	7.45	6.82	0.41	5.9
C	5.81	7.30	6.65	0.41	6.2
D	5.49	7.15	6.72	0.46	6.8
E	5.64	7.45	6.38	0.48	7.6
F	5.56	7.38	6.76	0.56	8.3
G	5.88	7.31	6.70	0.44	6.6
H	5.80	7.35	6.65	0.49	7.4
I	5.79	7.38	6.72	0.58	8.6
J	5.96	7.25	6.64	0.43	6.4
All houses			6.69	0.12	1.8

Table 3. Counts (log cfu/ml) of homo- and heterofermentative *Lactobacillus* spp. from various samples obtained from different sources

	Homofermentative			Heterofermentative		
vending houses	Mean	SD	%CV	Mean	SD	%CV
A	6.41	0.38	5.9	6.09	0.35	5.7
B	6.53	0.44	6.7	5.80	1.86	32.1
C	6.33	0.52	8.2	6.12	0.44	7.1
D	6.27	0.38	6.1	6.61	0.38	5.8
E	5.78	0.38	6.6	6.07	0.55	9.1
F	6.28	0.53	8.4	6.43	0.67	10.5
G	6.34	0.66	10.4	6.42	0.42	6.6
H	6.14	0.52	8.5	6.43	0.52	8.1
I	6.29	0.55	8.8	6.34	0.75	11.8
J	6.11	0.33	5.5	6.28	0.52	8.3
All houses	6.25	0.19	3.1	6.26	0.23	3.6

Although variations were not seen in counts of most samples within vending houses, it is worth noting that a good number of the samples did not yield any homofermenters, heterofermenters and, in certain cases, both (Table 4). Thus absence of variation is noted only in those samples where one or both of the groups of *Lactobacillus* spp. were counted. Total absence of one or both of these groups in some samples obtained from the same source indicated a notable variability in the product.

Table 4: Number of samples with undetectable homofermenters, heterofermenters or both

Vending house	No homo-fermenter	No hetero-fermenter	Both absent
A	4	9	6
B	7	9	3
C	5	11	3
D	10	11	6
E	10	8	4
F	7	11	6
G	7	9	4
H	1	8	1
I	9	6	1
J	10	11	9

Among the lactic acid bacteria, *Streptococcus* spp., *Pediococcus* spp, and *Leuconostoc* spp. were encountered frequently. They were among the dominant bacterial groups in 38%, 34% and 58%, respectively, of the samples collected from all vending houses (Table 5). Although these groups were important in relatively smaller number of samples, they did not show significant variation in their counts either within samples of the same source or among samples obtained from different sources. Exceptions are two samples within vending houses E and I for pediococci and *Leuconostoc* spp, respectively (CV >10%).

The spss outputs indicate that *'tej'* samples of all vending houses did not have significant differences between the mean values of total LAB, Homofermentative and heterofermentative LAB (ANOVA p, 0.193, 0.02, 0.027 respectively). So, the mean values of lactic acid bacterial population of *'tej'* samples in all the vending houses were similar with no significant difference.

Table 5 Counts (log cfu/ml) of Streptococci, Pediococci and *Leuconostoc* spp. in various samples from different sources.

Vending	Streptococci				Pediococci				*Leuconostoc*			
House	positive samples	X	SD	%CV	Positive samples	X	SD	%CV	Positive samples	X	SD	%CV
A	7/19	6.32	0.35	5.6	8/19	6.29	0.45	5.6	14/19	6.29	0.27	4.2
B	7/20	6.14	0.26	4.2	9/20	6.46	0.31	4.7	11/20	6.25	0.47	7.5
C	7/20	6.20	0.36	5.8	4/20	6.37	0.43	6.7	8/20	6.13	0.52	8.4
D	9/20	6.29	0.49	7.7	5/20	6.27	0.26	4.2	10/20	6.31	0.52	8.2
E	7/20	6.12	0.40	6.6	9/20	6.06	0.65	10.7	11/20	5.82	0.35	6.0
F	9/20	6.20	0.55	8.9	8/20	6.15	0.52	8.4	14/20	6.32	0.54	8.5
G	8/20	6.19	0.37	6.03	7/20	5.75	0.29	5.0	9/20	6.41	0.44	6.8
H	6/20	5.78	0.21	3.7	4/20	6.36	0.42	6.6	10/20	6.18	0.45	7.2
I	6/20	6.08	0.50	8.2	4/20	5.83	0.46	7.9	14/20	6.33	0.66	10.4
J	10/20	6.26	0.41	6.5	9/20	5.97	0.43	7.2	15/20	6.41	0.43	6.7
All houses	76/199	6.16	0.15	2.4	67/199	6.15	0.23	3.7	116/199	6.25	0.17	2.6

All the yeast isolates were asporogenous and fermentative. All of them were negative for the fermentation of L-rhamnose, salicin and lactose; positive for the assimilation of D-glucose and D-fructose; and negative for the assimilation of soluble starch. Lactose was assimilated only by *Kluyveromyces bulgaricus.*

The yeast isolates consisted of members of the genus *Saccharomyces,* which was the dominant yeast group and isolated from all, but one *'tej'* vending house. A total of 10 different species made up the yeast flora (Table 7). Of the 163 yeast strains isolated from the different samples, *Saccharomyces cerevisiae* was the most dominant (25.85%) followed by *Kluyveromyces bulgaricus (*16.33%), *Debaromyces phaffi* (14.97%), *Kluyveromyces veronae* (10.2%) and others of smaller significance. Samples from vending houses H, I and J consisted of four different yeast species, samples from house G consisted of 5 different species, and samples from house A contained six different species. Molds were not encountered in any one of the 199 sample analyzed in this study.

Table 7: Percentage (%) and Physiological properties of the yeast isolates at the time *'tej'* was ready for consumption (V = variable, + = positive, - = negative)

Species of yeast Isolates	Percent (%)	Fermentation									Assimilation												
		Glucose	Sucrose	Raffinose	Maltose	Lactose	Galactose	Trehalose	L- Rhamnose	Salicin	Mannose	Raffinose	Lactose	Trehalose	Soluble starch	Maltose	D-Fructose	Sucrose	D-Glucose	Galactose	L-Rhamnose	Salicin	D-Mannitol	Glycerol
Hansenula subpeliculosa	7.8	+	+	v	v	+	-	-	-	-	+	+	-	+	-	+	+	+	+	-	-	+	+	+
Kluyveromyces vanudenii	4.08	+	+	+	+	-	+	-	-	-	v	+	-	+	-	+	+	+	+	-	-	+	+	+
Saccharomyces cerevisiae	25.85	+	+	+	+	-	+	-	-	-	+	+	-	-	-	+	+	+	+	+	-	-	-	-
Kluyveromyces veronae	10.2	+	+	+	+	-	+	-	-	-	+	+	-	-	-	+	+	+	+	-	-	-	v	-
Kluyveromyces bulgaricus	16.33	+	+	+	+	-	+	-	-	-	+	+	+	v	-	+	+	+	+	+	-	-	-	v
Debaromyces phaffi	14.97	+	+	+	+	-	+	-	-	-	+	+	-	+	-	+	+	+	+	+	-	-	-	+
Saccharomyces rouxii	8.16	v	V	-	+	-	-	+	-	-	+	-	-	+	-	+	+	v	+	-	-	-	v	+
Saccharomyces norbensis	7.48	+	V	+	v	-	-	v	-	-	+	+	-	-	-	+	+	+	+	v	v	-	-	+
Endomycopsis burtonii	2.72	+	+	+	+	-	+	-	-	-	+	+	v	+	-	+	+	+	+	+	-	-	+	+
Pichia ohmeri	2.72	+	+	+	+	-	v	-	-	-	+	+	-	+	-	+	+	+	+	+	+	v	+	+

2. Biochemical properties of *'tej'*

The mean pH values of *'tej'* samples ranged from 3.47 to 3.98 (Table 8). Significant variations in pH values within *'tej'* samples collected from the same source was noted in vending houses B, C, D and J (CV >10%). There was, however, no significant difference in mean pH values of *'tej'* samples from the different vending houses (CV < 10%).

Table 8. Variations in pH values of *'tej'* samples collected from various sources

Vendin house	Min	Max	Mean	SD	%CV
A	3.15	4.01	3.47	0.32	9.1
B	3.35	4.90	3.95	0.51	12.79
C	3.26	6.00	3.98	0.63	15.92
D	3.45	4.88	3.82	0.42	10.96
E	3.24	4.07	3.63	0.25	6.81
F	3.23	3.99	3.68	0.25	6.99
G	3.02	3.96	3.58	0.30	8.28
H	3.07	4.25	3.80	0.32	8.48
I	3.41	4.32	3.82	0.30	7.94
J	3.14	4.41	3.87	0.39	10.22
All houses			3.76	0.16	4.2

The titratable acidity of *'tej'* samples ranged from values as low as 0.066% lactic acid to values as high as 0.258% lactic acid (Table 9). However variations in samples within a vending house was significantly high in all samples with CV values as high as > 18% in 7 of the 10 vending houses. In addition, variation in titratable acidity values among the various vending houses was also significantly high (CV >10%).

The chi-square values for titratable acidity and pH, 80.2 and 25.07, are both above the tabulated chi-square value (table 8) and both showed significant differences (ANOVA, $p \leq 0.003$ and $p < 0.001$ respectively) between their mean pH and mean titratable acidity in all the vending houses.

Table 9 Variations in titratable acidity (expressed as % lactic acid) in various *'tej'* samples collected from different vending houses

Vending house	Min	Max	Mean	SD	%CV
A	0.09	0.17	0.14	0.03	21.4
B	0.07	0.13	0.10	0.01	10.0
C	0.07	0.14	0.13	0.02	15.4
D	0.07	0.13	0.09	0.01	11.1
E	0.07	0.26	0.15	0.05	33.3
F	0.08	0.14	0.11	0.02	18.2
G	0.08	0.16	0.11	0.02	18.2
H	0.07	0.13	0.11	0.02	18.2
I	0.08	0.13	0.11	0.02	18.2
J	0.07	0.13	0.10	0.02	20.0
All houses			0.12	0.02	15.7

Six of the nine *'tej'* vending houses had samples with mean fusel oil contents of $\geq$ 20 g/100 liter. Variations within samples of *'tej'* from the same source was very highly significant (CV >50%) and even variations among vending houses was significant (CV >20%) (Table 10). The mean alcohol content of *'tej'* samples ranged from 6.98% to 10.29%. Variations within samples obtained from same vending house were significantly high with CV values exceeding 25% in nine of the ten vending houses. Variation of alcohol content of *'tej'* samples among the vending houses was also significantly high (CV > 10%).

The overall mean total alcohol content of *'tej'* of is 9.07% v/v with a range of 0.32 to 21.72% v/v.The mean alcohol content of samples of all the vending houses had higher calculated chi-square value than tabulated one. And so, all of the vending houses showed statistically significant difference (ANOVA, $p < 0.001$) between their mean alcohol contents.

According to the spss out puts , there is no statistically significant difference (ANOVA, $\rho > 0.086$) between all the vending houses in the mean fusel oil contents of their *'tej'* samples. Although, the overall mean fusel oil content (20.595g/100L) and the mean fusel oil value of *'tej'* samples in each vending house are very high values from the point of view of possible health risks.

The total protein content of *'tej'* samples collected from different vending houses varied from values as low as 0.06 mg/ml to values as high as 9.40 mg/ml. Mean values for vending houses A, B, C, and D were < 0.5 mg/ml, and those for vending houses E, H, I and J were between 1.4 and 1.9 mg/ml. Mean values for houses F and G was relatively higher (3.32 and 4.66 mg/ml, respectively). Samples from same source showed significantly high variation with CV values ranging from >40% to 107% (Table 11). Significantly high variation was also noted for samples between the different vending houses (CV = 84.8%).

Total carbohydrate content of the samples collected from all vending houses varied between 0.57 mg/ml and 5.85 mg/ml. Variations in samples within same source was significant but at lower levels of variability for vending houses A, B, C, G, H and I (Table 12). In vending houses E and J, the variation was at a higher level. Variations in mean total carbohydrate content of *'tej'* samples among vending houses was also significantly high (CV = 25.2%). Similar variations in reducing sugar content were also observed within samples of the same source or between vending houses.

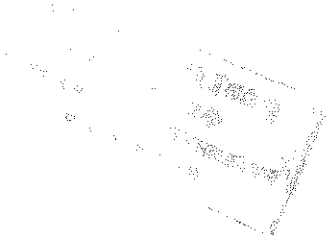


Table 10 Variations in fusel oil and alcohol content of *'tej'* samples collected from different sources

Vending houses	Fusel oil (g/100 l)			Alcohol (% v/v)		
	Mean	SD	%CV	Mean	SD	%CV
A	ND	ND	ND	10.29	2.97	28.9
B	22.91	11.71	51.11	10.17	1.46	14.4
C	13.92	10.99	78.96	8.33	2.08	25.02
D	13.58	9.82	72.31	10.08	3.01	29.89
E	23.47	17.17	73.15	9.57	2.64	27.58
F	19.80	14.69	74.19	9.25	3.08	33.29
G	15.49	12.74	82.25	8.39	4.50	53.62
H	27.38	20.86	76.19	10.04	3.79	37.83
I	25.72	13.55	52.68	6.98	2.35	33.64
J	23.12	13.90	60.12	8.12	2.41	29.64
All houses	20.60	4.86	23.60	9.12	1.06	11.60

'tej' samples had lipid content ranging from 0.03 mg/ml to 0.62 mg/ml. The mean content for most samples within same source was > 0.5 mg/ml, although the variation within the same source was enormous with coefficient of variation ranging from 50% to 170%, in eight of the ten vending houses (Table 12). In addition to this variation in samples within the same source, significant variation was also observed in mean lipid content between vending houses.

Table 11: Total protein, carbohydrate, lipid and reducing sugar content of *'tej'* samples

Vending House	Total protein (mg/ml)			Total carbohydrate (mg/ml)			Total lipid (mg/ml)Reducing sugar (mg/ml)		
	X	SD	%CV	X	SD	%CV	X	SD	%CVXSD%CV
A	0.39	0.17	43.6	1.49	0.42	28.2	1.34	0.52	38.80.580.2034.5
B	0.43	0.26	60.5	3.08	0.88	28.6	0.31	0.53	170.91.180.9782.2
C	0.33	0.22	66.7	2.81	0.80	28.5	0.56	0.38	99.72.091.5373.2
D	0.43	0.18	41.9	3.21	1.05	32.7	0.98	1.10	112.21.691.73102.3
E	1.47	1.02	69.4	2.99	1.51	50.5	0.64	0.38	59.41.361.3498.5
F	3.32	3.56	107.2	1.81	0.55	30.4	0.44	0.32	72.70.460.1123.9
G	4.66	2.87	61.6	2.10	0.61	29.1	0.36	0.26	72.20.991.14115.2
H	1.90	1.68	88.4	3.73	1.05	28.2	0.71	0.32	45.11.721.0460.5
I	1.62	1.32	81.5	3.52	0.96	27.3	0.87	0.44	50.61.711.6495.9
J	1.40	1.28	91.4	2.89	1.35	46.7	0.73	0.40	54.80.920.4447.8
All houses	1.60	1.35	84.8	2.76	0.70	25.2	0.69	0.30	42.71.270.5140.1

V. DISCUSSION

The aerobic mesophilic bacteria and the aerobic spore formers had very low incidence in the various samples collected from the different vending houses. *Coliforms* and members of the *Enterobacteriaceae* were not encountered in any of the samples. Such level of pH and acidity observed in the *'tej'* samples does not usually encourage growth or survival of various other groups of microorganisms unless they have the physiological adaptation to survive or thrive in such environments. This, however, would not marginalize the role of aerobic mesophilic bacteria, including *Coliforms* and members of *Enterobacteriaceae,* in the initiation or carrying out of *'tej'* fermentation as observed in other Ethiopian traditional alcoholic beverages' fermentations (Samuel Sahle and Berhanu A Gashe, 1991 ; Ketema Bacha et al., 1998).

In any spontaneous fermentation like *'tej'*, there is always a succession of microorganisms that play definite roles by contributing desirable or undesirable products. The physical and chemical environment of the fermenting media, together with the microbial antagonism are the determinant factors for the succession of different microbial groups through successive stages of fermentation. So, a microbial group could be eliminated, limited in number, or flourish differently at different stages in the course of fermentation. Since samples of *'tej'* were collected and analyzed at the time *'tej'* was ready for consumption, namely at the final stage of fermentation, one can not merely conclude that the absence of certain groups of microorganisms or dominance by others was indicative of thethe exclusive importance of that dominant group during fermentation.

Members of *Enterobacteriaceae* could contribute to acid production in the early stages of fermentation but could be inactivated beyond the pH that they can tolerate. This could also be due

to the bacteriostatic and bactericidal effects of the bacteriocins produced by the lactic acid bacteria (Sanni, 1992). It is also possible that yeasts also act as antagonists of other microorganisms through induction of environmental pH changes (DoCarmo – Sousa, 1969). Organic acids are excreted as the metabolic products of yeasts, and both acids and alcohols are inimical to other organisms.

The lactic acid bacteria are known to produce a variety of chemical compounds relative to fermentation conditions. Autolysis of some lactic acid bacteria appears to be a crucial step in the release of intra cytoplasm enzymes such as peptidases that produce free amino acids which are aroma precursors (Lepeuple *et al.* 1998). The low mean pH (3.47 to 3.98) of *'tej'* samples indicated that *'tej'* at the time it is ready for consumption is acidic at its best. This is the case where efficient lactic acid fermentation will produce a pH of 4 or less, a level at which many bacterial pathogens are inhibited and at which many bacteria die at a rate that increases with the ambient temperature (Jay, 1992).

The lactic acid bacteria, as studied by many researchers (Haas, 1960; Kleyn and Hough, 1971; Achi, 1990; Motarjemi and Nout, 1996; Casal *et al.* 1998;) are known to produce mixed acids by attacking sugars. They are known to produce a variety of chemical compounds relative to fermentation conditions. Their metabolic products contribute to the acidity as well as adding distinctive flavor and aroma to the fermenting material. Certain strains are responsible to spoilage by producing slime or rope in beer (Haas, 1960; Kleyn and Hough, 1971; Jay, 1992), causing sarcina sickness by producing just a small amount of diacetyl (0.2ppm or above), or cause acidity or turbidity or off flavor. But, there is considerable evidence that lactic acid fermentation inhibits the survival and multiplication of a number of bacterial pathogens (Sanni, 1992; Damelin *et al.*

1995; Motarjemi and Nout, 1996; McAuliffe *et al.* 1998; Ryan *et al.* 1998; Delibandhoesing *et al.* 1998). The counts of the various lactic acid bacteria groups did not vary between samples or among vending houses. However, as these groups of bacteria were dominant in some samples and undetectable in others, the presence or absence of their products in *'tej'* would result in variability in chemical components of the various *'tej'* samples obtained from the same source or from different sources.

The increase in total titratable acidity (as lactic acid) and fall in pH during fermentations of alcoholic beverages is said to be due to the production of lactic acid by lactic acid bacteria. Some examples for this phenomenon are in the fermentations of Indian jackfruit wine (Dahiya and Prabhu, 1977), Turkish boza (Hancioglu and Karapinar, 1997), Zambian *munkoyo* (Zulu *et al.* 1997), Zimbabwean *masvusvu* and *mangisi* (Zvauya, 1997). In commercial beer production, acidic pH increases the Quantity of extractables from hops (*Humulus lupulus*),and enhances the activities of enzymes and inhibits the growth of undesirable microorganisms (Keleyn and Hough, 1971). The low pH and the high acidity of Egyptian *Bouza* indicate a fermentation by lactic acid bacteria and the alcoholic fermentation is a yeast fermentation (Morcos, 1977). In the fermentation of certain wines such as the Burgundies, the high malic acid content of the must is converted to the weaker lactic acid by lactic acid bacteria and thus the total acidity is lowered (Postgase, 1992).

Microbially produced lactic acid is usually a mixture of the L (+) and D (-) isomers of lactic acid. As the latter can not be metabolized by humans, excessive intake can result in acidosis (Motarjemi and Nout, 1996), which is a disturbance in the acid – alkali balance in the blood. Other than mixed acid products, lactic acid bacteria frequently produce biogenic amines such as histamine and tyramine in a variety of feeds and beverages (Damelin *et al.* 1995).

The mean values of the total LAB, homofermentative, and the heterofermentative lactic acid bacteria were similar (ANOVA, $p > 0.019$) between all the vending houses (Table 6). So, all the vending houses didn't show statistically significant difference between their mean values in their lactic acid bacterial populations.

Numerous lactic acid bacteria and yeast strains are being used for commercial wine fermentation, beer brewing, and bakery products, dairy products, fermentation of fish and meat products and fermented plant products (Querol *et al.* 1992; Jay, 1992; Johanson, 1995; Damelin, 1995). Souring process by lactobacilli in the first phase of kaffir beer fermentation differs it from European beers (Ekundayo, 1969). Both yeasts and bacteria have association in the fermentation of many different feeds and beverages (Adegoke and Babalola, 1988; Zulu *et al.* 1997). Yeasts not only provide humans with the first biotechnologically produced food such as wine, bread, and fermented milk products, but they are also responsible for food spoilage, and some spp are of medical importance (Kummerle *et al.* 1998). Yeast is one of the most nutritious of foods, being rich in protein and vitamin B group and a reasonable Quota of fats (Postgate, 1992).

Among the yeast isolates, it was only the species of the genus *Saccharomyces* that were isolated from samples of almost all the vending houses. Yeasts of the genus *Saccharomyces* were considered by Vogel and Abeba Gobezie (1977) to be responsible for the conversion of sugars to ethanol in *'tej'*. High sugar concentrations and high specific growth rates trigger alcoholic fermentation by *Saccharomyces cerevisiae* even under fully aerobic conditions (Vanhoek *et al.* 1998). Similar to findings in this study, the dominant organisms in Phillipine *basi* were *Saccharomyces*, *Endomycopsis* and lactic acid bacteria (Sanchez, 1977; Tanimura *et al.* 1978b).

Some investigators have detected traces of lactic acid in the alcoholic fermentation products of yeasts (Nord and Weiss, 1958). Lactic acid is one of the organic acids used by many yeasts and considered to be of special value for taxonomic studies (Morris, 1958). Species of *Saccharomyces* and *Pichia*, which relatively assimilate few carbon compounds, are abundant in fruit juices, sugary plant exudates and other material rich in simple sugars (DoCarmo – Sousa, 1969). Certain spp of *Pichia* and *Candida* have the ability to grow on lactic acid produced during the primary fermentation of brined olives (Ingram, 1958).

Hansenula subpeliculosa was isolated from samples of *'tej'* from five vending houses. According to Park *et al.* (1977), *Saccharomyces cerevisae* and *Hansenula* spp were responsible for alcohol production and flavor development respectively in the fermentation of Korean *Yakju*. *Hansenula* can also cause disease in fruit wine by affecting its flavor and by causing turbidity or slimy consistency by forming a film on the surface in the presence of ample oxygen (Lund, 1958).

Endomycposis burtonii was another yeast species isolated from *'tej'* samples. The yeast involved in the Indian jackfruit wine fermentation resembled *Endomycopsis* (Dahiya and Prabhu, 1977). *Endomycopsis* was also one of the dominant yeasts in Philippine *basi* (Sanchez, 1977; Tanimura *et al.* 1978), and hydrolyzes steamed rice (starch) to maltose and glucose and then ferment the sugar to ethanol and organic acids which provide flavor and aroma in the fermentation of Indonesian *tape Ketan* (Ko, 1977; Yeoh; 1977b).

Saccharomyces spp being dominant and followed by spp of *Kluyveromyces* and spp of *Endomycopsis* together make up more than three-quarters of the isolates. In this study, there was no vending house, which consisted of members of only a single yeast genus. Samples from five of

the vending houses contained yeast isolates belonging to two to three yeast genera, while the other five contained four to six genera of yeast isolates. Thus depending on number and type of yeast species, the chemical composition of the various products showed significant variations within samples of the same source and among vending houses.

The mean yeast populations among samples of all the vending houses are the same and all the vending houses have no significant differences (ANOVA p, 0.612) between their mean values in their yeast populations.(Table6).

Similar to observations in this study, the coexistence and free proliferation of lactic acid bacteria and yeasts was observed in the study of many beverages. It was observed in *masvusvu* (zvauya *et al.* 1997), *pulque* (Pedersen, 1979) *kumsis* and *kefir* (Pedersen, 1979), kenyan *uragua* (Harkishor, 1977), Philippine *basi* (Harkishor, 1977), palm wines (Plat and Webb, 1964: Faparusi, 1977), and Egyptian *bouza* (Morcos, 1977) etc. Theircoexistence is a common occurrence in food and beverage fermentation .

When compared to the others, the mean titratable acidity, as lactic acid, of *'tej'* was less than the average acidity of honey wines of Eastern Europe, although there was an overlap in the range in both types of drinks (Steinkraus, 1983). On the other hand, the range for the mean acidity of *'tej'* is wider than that for various meads (Steinkraus, 1983).Here *'tej'* is likely more variable than meads in terms of the relative width of their ranges. The range for the mean acidity of *'tej'* again is wider than that for the honey wines in the US market (Steinkraus, 1983) and also more than the acidity for the Mexican *pulque*, (Gonclaves de Lima, 1975). But, the mean acidity of *'tej'* is below the average acidity of Phillipine *basi* (sanchez, 1977; Tanimura *et al.* 1978b). Palm wines from

west Africa have a narrow range for the average acidity(Bassir, 1968) than that for *'tej'*. So, the mean acidity of *'tej'* being similar or close to that of honey wines of Eastern Europe, is in between the average acidity of palm wines from West Africa and Phillipine *basi*.

In comparison to the mean pH values of other beverages, the mean pH value of *'tej'*, falls in the range for the pH of meads (Steinkraus, 1983); Philippine *basi* , (Sanchez, 1977; Tanimura *et al.* 1978) ; Kenyan *muratina*, (Tanimura *et al.* 1978b) ; Mexican *pulque*,(Gonclaves de Lima, 1975) and Indian jackfruit wine, (Dahiya and Prabhu, 1977). But, the overall mean value for the pH of *'tej'* is slightly above the average and beyond the pH range for the honey wine of US market (Steinkraus, 1983). Generally, *'tej'* in comparison to other wines, is as acidic as honey wines of Eastern Europe and the US market, palm wines and sugarcane wines cited above. Acid and alcohol are two primary products of fermentation related to preservation (Steinkraus, 1983). Moreover, wine owes its microbiological stability to its acidity and alcohol content which permit a certain conservation without the use of highly specific techniques (Ribereau – Gayon, 1972). The continuous development of acidity in palm wine from West Africa disturbs the alcohol – acid ratio that contributes to the taste (Nyako, 1977).

Different fermented alcoholic beverages contain comparable amounts of alcohol as in *'tej'*. Philippine *basi* contained 9.4 to 15.4% (Sanchez, 1977; Tanimura *et al.* 1978a). Tanzanian *wanzuki* contained 8.5% (Nikander *et al.* 1991). Kenyan *muratina* 3.9 to 13.4% v/v (Tanimura *et al.* 1978b).

Fusel oils are produced by the actions of enzymes on the amino acids present in the fermentation medium. They are produced catabolically and/or anabolically from sugars via the pathways through which amino acids are synthesized (Spanyer and Thomas, 1957 Purchase, 1969; Jounela-Erikson, 1978; Reilly, *et al.* 1979; Nikannen and Suomalainen, 1983; TerSchure *et al.* 1998).

Saccharomyces cerevisiae is one of the few yeast spp that form the highest amount of fusel alcohols and fatty acid esters which contribute to the aroma of wines (Ribereau – Gayon, 1972). It is to be noted here that the majority of the yeast isolates (41.50%)are members of *Sacccharomyces.* Valine, Leucine, and isoleucine were used as precursors of Isobutyl, active amyl and isoamyl alcohols respectively by *Saccharomyces cerevisiae* (Spanyer and Thomas, 1957). Fusel oil came from the old German Word 'fousel', meaning bad spirit (Webb and Ingragam, 1963) due to its unpleasant odor.

Fusel oil is a collective name for isopropyl alcohol, asym-isoamyl alcohol or pentanol- 2, tert-buytl alcohol or trimethyl carbinol, ehtyl dimethyl carbinol, 1,3-propane diol, 1,2- propane diol, Poly (Vinyl) alcohol (Gibel *et al.* 1968; Purchase, 1969; Reilly *et al.* 1979;).

The preponderance of fusel oil in *'tej'* samples could be related to the dominant *Saccharomyces* from the yeast isolates. Other yeast species of *Hansenula, Debaromyces, Pichia* etc. are also said to produce polyhydric alcohols, such as butanediol, that are responsible for flavor development (Hackenhull, 1968). The pungent aromatic odor of *'tej'* could be attributed to its high fusel oil content and probably some other chemical contents. *Saccharomyces cerevisiae*, in its fermenting culture, along with ethanol and carbon dioxide produce a variety of low molecular-weight flavor compounds including fusel alcohols, diacetyl, esters, organic acids, organic sulfides, and carbonyl compounds (TerSchure, *et al.* 1998).

Fusel oils produce narcosis, intoxication and poisoning with respiratory failure, more readily than ethyl alcohol, being about five times as toxic (Locket, 1957). They may play a role in alcoholism and may act as putative carcinogens (Nikander, *et al.* 1991). Fusel oils are toxic, and their toxicity depends on the type of individual fusel oil component and route of administration, i.e. whether they are introduced orally, intravenously, intraperitonially or cutaneously, etc. into the body (Purchase, 1969). The toxic effect of aliphatic alcohol is a narcosis that acts on the central nervous system. Toxicity in general increases with the number of carbon atoms because, a high ratio of hydrocarbon proportion is obtained and this favors its solubility in the lipoid of the nervous system (Monick, 1968). Ingestion of 0.5g fusel oil in humans resulted somnolence, headache, and throat irritation. Larger quantities have caused diarrhea, nausea, vomiting, delirium, and coma. Tertiary amyl alcohol has caused severe intoxication, and contributed to death when an overdose was given by mistake in the form of an enema (Monick, 1968).

Some home produced but commercially available alcoholic drinks in Africa are known to contain high amounts of fusel oils. 280-320mg/L *wanzuki* of Tanzania (Reilly, *et al.* 1979) 227mg/ L in Bantu beer Purchase, 1969) 209 ppm and 1839 ppm (maximum) in Ethiopian. *'tej'* and *Katikala* (Alemu Fite, *et al.* 1991), 411 ppm/L in *chibuku* of Tanzania (Reilly *et al.* 1979).

The mean fusel oil contents of *'tej'* (20.595g/100L) is above the average fusel oil content of *chibuku* of Zambia,150ppm(Reilly *et, al.*, 1979)also above isobutanol content (140mg/L) of *wanzuki* (honey wine) and *tembo-mnazi*(palm wine) of Tanzania (Nikander *et al.* 1991); Mexican *pulque* ,30 to 100mg/L, (Gonclaves de Lima, 1975); British commercial beers, 100ppm (Reilly *et al.* 1979); *'tej'*,104ppm, from three different regions of Ethiopia (Alemu Fite *et al.* 1991) and German white wine, 11mg/L (Nykanen and Suomalinen, 1983).

More over, in comparison to some other alcoholic beverages, the mean fusel oil content of *'tej'* is more than isobutanol content of Japanese special class brandy (20mg/100ml), isobutanol content of Canadian whisky (9mg/100ml) while it is slightly lower than that of Japanese special class whisky, 23mg/100ml (Akiyama *et al.* 1978).The mean fusel oil content of *'tej'* again is a bit lower than fusel oil content of Bantu beer(227mg/L) which differs from other ordinary beers in having a higher fusel oil content(Purchase *et al.* 1969), and a bit further lower than fusel oil content of South African white wine, 234ppm(Reilly *et al.* 1979).Of the major components of fusel oils, amyl alcohols are used in many industries as solvents for a variety of resins, as additives for lubricating oils, hydraulic fluids, as anti foaming agents, riffle-bore cleaners, etc.(Monick, 1968).

The preponderance of fusel oils in many fermented alcoholic beverages is attributed to a prolonged fermentation. Commercial and laboratory prepared *chibuku* of Zambia from maize or sugar and added yeast, with a short fermentation period contained lower levels of fusel oil than *chibuku* collected from village. But, by allowing natural fermentation by acid producing *Bacilli* and naturally occurring yeast, the prolonged fermentation (as long as 7 days) of the mash encouraged the formation of increased amounts of fusel oils from amino acids (Reilly *et al.* 1979).Fermentation of *'tej'* takes 2 to 5 or more times the period needed for the preparation of *chibuku.* The fusel oil contents of the Lebanese *arak* did not vary in tandem with the levels of their amino acid precursors. According to Dagher and Ruhayyim (1975), their variability was attributed to the variability in yeast population responsible for the fermentation.

The high alcohol of wine is tolerable because of wine's high acidity (due to tartaric acid in grapes), which in turn is counterbalanced by the sweet taste (Noble, 1978). The most desirable quality in palm wine is its sweet alcoholic flavor, but the slightly sour taste produced by combination of small amounts of lactic acid is also desirable (Okafor, 1972b). The slightly sour taste of *'tej'* is also preferred by consumers and highly sweet *'tej'* is disapproved as "*birz*"or "*beteha*", a *'tej'* that has not yet developed the required alcoholic content and flavor.

The overall mean total alcohol content of *'tej'* of is 9.07% v/v with a range of 0.32 to 21.72% v/v. The mean alcohol content of samples in all the vending houses or in samples from same vending house showed great variability possibly due to differences in number and types of yeast flora. Similar results were observed in the variability of *'tej'* collected from three different regions of Ethiopia. In these samples of *'tej'* the total alcohol and fusel oil became more pronounced between the regions than within (Alemu Fite *et al.* 1991).

Honey wine of Eastern Europe had an alcoholic content of 6.4 to 16.6% v/v (Sarin, 1921) while meads had 6.6 to 14.2% v/v (Steinkraus, 1983). Honey wines of the US market were reported to have an alcoholic content of 12.2 to 20.8% v/v (Steinkraus, 1983). Vogel and Abeba Gobezie reported that if fermentation of *'tej'* goes to completion, the final alcohol content would be 7 to 13%v/v (Steinkraus, 1983). Belachew Desta (1977) in his survey of alcoholic content of some traditional beverages of Ethiopia, reported the ethanol content of traditional *'tej'* to range from 13 to 13.73%v/v. Alemu Fite *et al.*(1991) had reported 6.2% as a mean ethanol value of *'tej'* samples from three different regions (Gojam, Debre-Berhan and Addis Ababa) of Ethiopia. The *'tej'* samples they collected from Addis Ababa had an average ethanol content of 7.5%v/v with a range of 6.6 to 8.4%v/v.

In comparison to the above beverages, the mean alcoholic content of *'tej'* in this study is above that of *'tej'* from Addis Ababa as reported by Alemu Fite etal.(1991), and it falls in the range for that of meads, (Steinkraus, 1983); Coconut toddy (Bassir, 1968), and other samples of *'tej'* (Steinkraus, 1983). But, the mean alcohol content of *'tej'* in this study is below the mean alcohol content of honey wines in the US market (Steinkraus, 1983) and *'tej'* as reported by Belachew Desta (1977).

The mean protein content of *'tej'* is well above the protein nitrogen in Nigerian palm wines(toddys) (Bassir, 1968; Faparussi and Bassir, 1972 a,). The primitive wines and beers contain small amounts of proteins and amino acids, which contribute to the protein nutrition of consumers(Steinkraus, 1983). Indian jackfruit wine contains protein well above the protein content of *'tej'* (Gonclaves de Lima, 1975). But, the total nitrogen content of Philippine *basi* , 1% (Sanchez, 1977; Tanimura *et al.* 1977) and Malaysian coconut toddy, 0.1%(Merican, 1977) are far below the mean protein content of *'tej'*.

According to the spss computer package outputs (table 10), statistically significant difference (ANOVA, $P < 0.001$) is observed between the mean values of all the vending houses in the protein content of their *'tej'* samples.

The mean total carbohydrate content of *'tej'* is far below the residual sugar content of honey wines of Eastern Europe, (Steinkraus, 1983) and below the residual sugar content of honey wines from US market (Steinkraus, 1983).The mean reducing sugar content of *'tej'*, 1.26mg/ml, is also below those of honey wines from US market (Steinkraus, 1983); Philippine *basi* (Sanchez, 1977; Tanimura *et al.* 1978); Mexican *pulque* (Gonclaves de Lima, 1975); and Indian jackfruit wine (Dahiya and Prabhu, 1977).

The overall mean total carbohydrate and reducing sugar contents of *'tej'* are found to be 2.76mg/ml and 1.26mg/ml respectively. Statistically significant difference (ANOVA, $P < 0.001$ for both) was observed between the mean values of all the vending houses in their mean total carbohydrate and reducing sugar contents respectively (table 10).There was a statistically significant difference (ANOVA, $P < 0.001$) observed between the mean total lipid content of all the vending houses. The overall mean lipid content of *'tej'* was 0.683mg/ml.

As can be seen from table 10, the spss output shows that generally all the chemical properties had higher chisquare values and lower ρ-values. Calculated chisquare values for the mean chemical properties of all vending houses, except for fusel oil, were higher than tabulated (23.59) chisquare value at 9 degrees of freedom. Generally, all the vending houses showed significant difference (ANOVA, $\rho \leq 0.003$ for all) between their mean values in their chemical properties except in fusel oil.

VI. CONCLUSION AND RECOMMENDATIONS.

'tej' is a widespread home industry of considerable social and economic importance. Preparation of African traditional beverages are still traditional family arts and the fermentation is spontaneous with mixed culture, therefore, variation in the quality and stability of products are the recurring disadvantages (Sanni, 1993).Like other African beverage the fermentation process of *'tej'* is also by uncontrolled fermentation, quantity of substrates, physical conditions, and duration of fermentation. More over, concoction and adulteration (with readily fermentable sugar sources) practices to increase alcohol content and potency are widely applied. All these factors contribute to the variability of the product.

This study indicated that generally there is significant variability of *'tej'* at the time it was ready for consumption, both within and between all of the vending houses based on various microbiological and chemical properties. Samples of *'tej'* from same vending house were significantly different from each other in their microbiological and chemical properties. Also samples of one vending house are significantly different from samples of other vending houses in their microbiological properties and chemical properties as observed in this study.

Some of the basic raw materials for *'tej'* preparation may not be different and the skills for the preparation of *'tej'* had passed from generation to generation informally and orally, so there may not be much pronounced difference in skills in the preparation of *'tej'*. Now a days, however, it seems unlikely that there is *'tej'* prepared from a pure honey alone. Different amounts of sugar are added to the fermentation vats. Concoction practices in order to increase the alcohol content and potency of *'tej'* are the top-secrets in *'tej'* preparation. Roots, barks, leaves and stems of herbal plants are used for this purpose. Some vending houses are said to add porridge prepared from

powdered maize, into the fermentation vats and others add *katikalla,* an alcoholic spirit (personal communications).

Microbiological and chemical variability of *'tej'* could be attributed to the spontaneous fermentation, since *'tej'* fermentation depends on the microflora naturally present in the substrates on utensils and equipments used. The different metabolic products of these randomized microflora at different stages, the physical and chemical environments and duration of fermentation and concoction practices have influence on the succession of microorganisms during fermentation and consequently result in microbiological and chemical variability of *'tej'* at the time it was ready for consumption. After investigation of this variability of *'tej'* from some vending houses in Addis Ababa, the following could be recommended:

1 Research on optimal production conditions and establishing standards with an elucidation of factors such as substrates, physical and chemical conditions, nutritive changes and other processes involved in the preparation of *'tej'* is recommended. *'tej'* is a popular national drink among people of different social strata in Ethiopia. More study on starter cultures and optimization of process and fermentation conditions could result in a standard product with wider acceptance.

2 Large number of population in most parts of the country consumes *'tej'*. From the point of view of public health, investigations on the mechanisms of production and means to avoid or reduce the preponderance of harmful contents are necessary.

An introduction of even a little technological refinements to tackle this problem could be envisaged by researchers. Controlled use of metabisulfite in the production of Indian jackfruit

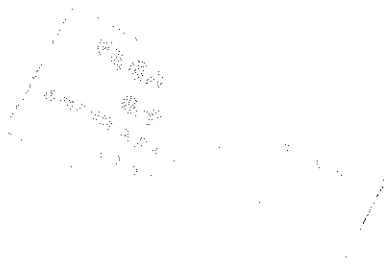
wine helped in the control of wild yeasts (Dahiya and Prabhu, 1977). In the production of chibuku of Zambia, seeding the fermentation mixture with a large yeast inoculation or adding an ammonium salt reduced the amount of fusel oil produced at least by half(Reilly *et al.* 1979).Research is a prerequisite to gearing such simple technological refinements in to action. Regardless of their ability to produce ethanol, fusel oil production(aerobically or anaerobically)appears to be a general property of yeasts(Webb and Ingraham, 1963). Even if fusel oil formation seems unavoidable, seeking ways to reduce amounts produced based on optimization of substrate, physical and chemical conditions require attention and intensive research.

3. Because of the wide-spreading and seriousness of alcohol-related problems, the World Health Assembly in its resolution WHA 36.12at the World health Assembly in 1983 emphasized the necessity for countries to develop comprehensive national alcohol policies. Control and supervision of local brews in Nigeria and some African states, such as legislation to control production of alcoholic drinks in Tanzania (Nikander *et al.* 1991) is also another indispensable example. The quality of traditional homemade beverages is largely outside social control and surveillance, so, control in production and supervision with the development of comprehensive national alcohol policy are recommended.

4. Prolonged and different periods of fermentation might be assumed to account for the high amount and variability of fusel oil contents with the randomized microflora of an unknown yeasts, acid producing bacilli, and other microorganisms inhabiting the must from different sources. Further research on other possible harmful components and on individual fusel oil contents of *'tej'* is required to estimate whether they are found in *'tej'* above or below their maximum allowable

concentrations. This also needs assessing factors responsible for the high magnitude of variability in its microbiological and chemical properties.

5. In modernization, acceptability vis- â-vis the cost of the product should be the main point. Refining, bottling or canning may interest those with more purchasing power unless it remains a beverage of lower income group with a ready, hot, and wide market if acceptable by this social stratum. Further researches are required to pave ways to bring it to this stage



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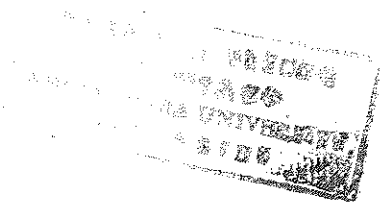

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