DETERMINATION AND COMPARISON OF ALCOHOLS CONTENT IN LOCAL ALCOHOLIC BEVERAGES MADE OF DIFERRENT CEREALS

BY ABERRA LEMMA

A THESIS SUBMITTED TO THE DEPARTMENT OF CHEMISTRY SCHOOL OF APPLIED NATURAL SCIENCE

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMEN FOR THE DEGREE OF MASTER'S IN CHEMISTRY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

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APPROVAL OF BOARD OF EXAMINERS

We, the undersigned, members of the Board of Examiners of the final open defense by Aberra Lemma have read and evaluated his thesis entitled "**DETERMINATION AND COMPARISON OF ALCOHOLS CONTENT IN LOCAL ALCOHOLIC BEVERAGES MADE OF DIFFERENT CEREALS"** and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in Chemistry.

_____ _____ _____

Advisor Signature Date

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DECLARATION

I hereby declare that this M.Sc. Thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Aberra Lemma

Signature:_____

This M.Sc. Thesis has been submitted for examination with our approval as thesis advisors.

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ADVISOR'S APPROVAL SHEET

To: Chemistry Department

Subject: Thesis Submission

This is to certify that the thesis entitled "DETERMINATION AND COMPARISON OF ALCOHOLS CONTENT IN LOCAL ALCOHOLIC BEVERAGES MADE OF DIFFERENT CEREALS" submitted in partial fulfillment of the requirements for the degree of Master's of Science in Chemistry, the Graduate program of the department of Chemistry, and has been carried out by Aberra Lemma Id. Nº GSU/0484/06, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby he can submit the thesis to the department.

_____ _____ _____

Name of major Advisor Signature Date

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Name of Co-Advisor Signature Date

DEDICATION

This thesis manuscript is dedicated to my beloved wife Hababo Areda for her concern, love, patience, affection and support.

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LIST OF ABBREVIATIONS

ABV	Alcohol content By Volume
ABW	Alcohol content By Weight
AOAC	Association of Official Agricultural Chemists
BMTC	Barley-Maize Tella Composite
BTC	Barley Tella Composite
MTC	Maize Tella Composite
PAC	Purchased Arekie Composite
PTC	Purchased Tella Composite
DEAS	Draft East African Standard
EAC	East African Community
EAS	East African Standards
ECAE	Ethiopian Conformity Assessment Enterprise
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
LAB	Lactic Acid Bacteria
RTD	Ready To Drink
SG	Specific Gravity
US	United States
WHO	World Health Organization

ABSTRACT

One of the most consumed fermented alcoholic beverages is tella, which is made mostly with barley but wheat, maize, sorghum, and teff are utilized depending on the region. Tella fermentation was carried out using traditional methods.The main objective of this work was the direct determination and comparison of ethanol and methanol level in traditional fermented alcoholic beverages of home prepared tella samples from different cereals and tella sample purchased from vending house using Alcolyzer beer method and GCFID method respectively. The ethanol content of Arekie sample was determined using pycnometer method.). The average ethanol content of Tella samples ranged from3.00% to 6.91%v/v and pH ranged from 4.41 to 5.43. In all of the tella samples there is significant variation in ethanol content and however, in pH values there is no significant variation between the tella samples BTC and MTC, MTC and BMTC, whereas there is significance variation between tella beverages BTC and BMTC, BTC and PTC, MTC and PTC as indicated statistically with one way ANOVA (at p =0.05) level.The average alcohol content of arekie was found to be 49.02%v/v which is higher than the reported value of terra arekie 37.22%v/v or even than that of dagim arekie 48%v/v. The GC analysis of tella and arekie indicates that there is methanol in all of the samples with the highest concentration in barley tella and least in the mixture of barley and maize tella beverages.

Key words: Beverages, Ethanol, Methanol, Tella, Alcolyzer,

1. INTRODUCTION

1.1 Background of the study

There are numerous traditional alcoholic beverages which are locally produced and consumed among native peoples of many countries around the world. Such types of drinks are very commonly produced in a variety of ways as home-made beverages in many African countries (WHO, 2004).Alcoholic beverages have been widely consumed since prehistoric times by people around the world, seeing use as a component of the standard diet, for hygienic or medical reasons, or recreational purposes, and for other reasons (Siyum, 2015). Alcoholic beverages of plant origin represent a vast diversity of products (Tamang, 2010).However; in general, alcoholic beverages can be classified in to three main categories: wines, beers, and spirits. This classification is based on production methods: 1) by mono-fermentation; (2) by malting and fermentation; and (3) by distillation after fermentation. The development of distilled alcoholic beverage is associated with the advent and improvement of distillation technique. Traditional fermented beverages are those that are indigenous to a particular area and have been developed by the local people using an age-old technique and locally available raw materials (Abegaz *et al*., 2002). Home-brewed beverages have several names mostly reflecting the areas where they are produced but most of them are produced in almost similar ways by fermentation and distillation of grain cereals, fruits and/or vegetables (Shale *et al*., 2014). Distillation is a more complex time consuming method requiring specialized equipment to produce potent alcoholic beverages. The preparations of many traditional fermented foods and beverages remains as a household art. They are produced in homes, villages and small scale industries (Solange *et al*., 2014). In Ethiopia, the traditional cereal based fermented beverages and foods are still prevailing in both rural and some urban communities. In our country, different types of traditional alcoholic beverages such as Tella, Korefe, Shameta, Arekie, Borde, Tej and Kineto are

produced and consumed. Ethanol is the only type of alcohol that can be consumed (drunken). Most farmers engage in alcohol production not only as a means to generate income to earn a living, but also due to the high level of demand and use in diverse ways by consumers across the globe (Glithero et al. 2013). Commercial ethanol for consumption is prepared from various forms of starch and sugars by fermentations. The quality of alcoholic beverages may impact on health and mortality, for instance when home-made or traditionally produced alcoholic beverages are contaminated with methanol or other very toxic substances, such as disinfectants (WHO, 2014). The complications and chronic diseases or ill health such as blindness, dizziness, and respiratory diseases etc., associated with drunkenness suggested that there may be the presence of methanol and other toxic substance in the drink that affects the health of the people (Lachenmeier 2007). There are three main direct mechanisms of harm caused by high alcohol consumption in an individual.

These are:

i. Toxic effects on organs and tissues;

ii. Intoxication, leading to impairment of physical coordination, consciousness, cognition, perception, and behavior.

iii. Dependence, whereby the drinker's self-control over his or her drinking behavior is impaired. In addition to the causal relationships between alcohol consumption and disease and injury categories, a strong association exists between alcohol consumption and HIV infection and sexually transmitted diseases (WHO, 2014). Ethanol affects the central nervous system, gastro-intestinal tract, cardiovascular system, endocrine, liver, lipid metabolism, fetal development, and has immune suppression activities. Alcoholic strength is the term used to denote the measure of the amount of ethyl alcohol (ethanol) in solutions such as beer, cider, kombucha,malt-beverages, RTD cocktail mixes, wines

and spirits/liqueurs. It may be reported as percent by mass of solution (% weight/weight – w/w or mass/mass - m/m) or as percent by volume (% volume/volume – v/v). These values are expressed at either 15.56 °C or at 20 °C depending on country, regulatory authority or other local requirements. Alcohol may be accurately determined via: Gas Chromatography (GC) and Liquid Methods (Including High Performance or High Pressure Liquid Chromatography (HPLC)), Density and/ or Specific Gravity Measurement (Spedding, 2015).

1.2 Statement of the problem

In 2012, of all global deaths 5.9% were attributable to alcohol. Overall, about 3.3 million deaths in 2012 are estimated to have been caused by high alcohol consumption. This corresponds to 5.9% of all deaths, or one in every twenty deaths in the world (7.6% for men, 4.0% for women (WHO, 2014). No particular broad disease category stands out. The highest numbers of deaths are from cardiovascular diseases, followed by injuries (especially unintentional injuries), gastrointestinal diseases and cancers (WHO, 2014). According to World Health Organization (WHO), the harmful use of alcohol causes an estimated 2.5 million deaths every year, of which a significant proportion occurs in the young. There is also emerging evidence that the harmful use of alcohol contributes to the health burden caused by communicable diseases such as tuberculosis and HIV/AIDS (WHO, 2004; WHO-AFRO, 2010). There are several damaging effects of alcoholism such as mental problems, job trouble, frequent block outs, loss of control etc. (Olandeinde et al, 2002). Ethanol affects the central nervous system, gastro-intestinal tract, cardiovascular system, endocrine, liver, lipid metabolism, fetal development, and has immune suppression activities. The distinctive volatiles, which give characteristics to distilled alcoholic beverages, are affected by many variables such as raw materials, flavor additives, and the processing steps, which include fermentation, distillation and aging (Cole and Noble, 2003). The basic substrates used for the preparation of most of the alcoholic beverages; particularly

home-processed ones are adequate media for the growth of many types of microorganism (Pederson, 1979). Considerable evidences demonstrate that some of the popular traditional alcohol drinks produced in Africa are contaminated with bacteria (Holzapfel, 2002) and contain harmful impurities and adulterants (Sanni, 1993). For example, member of the bacteria types such as Staphylococcus, Escherichia, and Salmonella have been reported to be present in a number of traditional food products and beverage drinks in Africa (Lues et al., 2011). Therefore, unless fermentation conditions are optimized in order to obtain consistent products, the complex micro flora implicated in spontaneous fermentations, are unpredictable and they lead to the variability in the quality and stability of the product. The level of methanol and the main volatile compounds (higher alcohols, esters, aldehydes, volatile acids) of distilled beverages are one of the most important quality and safety factors that should be known and controlled by producers and legal authorities. Because these are the common contaminants of traditional alcoholic beverages. The initial symptoms of methanol intoxication include central nervous system depression, headache, dizziness, nausea, lack of coordination, and confusion. Sufficiently large doses cause unconsciousness and death (Kruse, 2012).Excessive alcohol consumption was responsible for 1.8 and 2.5 million deaths worldwide, in the years 2004 and 2011, respectively. In 2000, 140 Kenyans died, many went blind, and others were hospitalized after consuming an illegal brew from sorghum, maize, or millet, called kumi kumi (Mureithi, 2002). According to the estimates by an international group of experts, alcohol consumption was responsible for 13.6% of premature mortality cases among men aged 20 to 64 years in Poland, 16.3% in the Czech Republic, 22.8% in Lithuania, and 25.2% in Hungary([Rehm et al, 2007). It was implicated in over 60 diseases, accidents and injuries and a contributing cause in 200 others. Overall, alcohol is a cause of more than 60 different health conditions and, for almost all conditions, heavier alcohol use means higher risk of disease or injury (Rehm,et al;2010, Room, et al; 2005). About 130 persons died in some India villages in 2011 linked to poisonous ethanol consumption.

In Czech Republic, 127 persons were poisoned from contaminated alcohol, out of which 42 died (Vaskova, 2013). In 2014, the World Health Organization (WHO) alerted that there have been increasing outbreaks of methanol poisoning in several countries including Kenya, Gambia, Libya, Uganda, India, Ecuador, Indonesia, Nicaragua, Pakistan, Turkey, Czech Republic, Estonia and Norway. The size of these outbreaks ranged from 20 to over 800 victims, with case fatality rates of over 30 % in some cases (WHO, 2014). In Nigeria, between April and June 2015, a total of 89 persons died following the consumption of locally produced ethanol beverage called kaikai/ogogoro/apeteshi or illicit gin. While investigation is ongoing on the source/origin of methanol in the beverage, the Federal Government of Nigeria (FGN) placed a ban on the production, sale, distribution and consumption of locally fermented beverage in Nigeria (Elijah, 2016). WHO (2014) reported that blood methanol concentration above 0.5mg/mL is associated with severe toxicity, whereas concentration above 1.5–2.0mg/mL causes death in untreated victims. Much alcohol consumption is also associated with other serious social harms including violence, child neglect and abuse, and absenteeism from work. The health, especially of young people is increasingly being threatened by dangerous patterns of alcohol consumption in recent years (, 2004; WHO, 2011).Therefore, it is the aim of this research to determine the level of ethanol and methanol in each cereal grain beverages, identify the cereals' beverages with the lowest alcoholic content and the presence of some higher alcohols which are risk of health and also to answer questions that mostly rose by consumers of traditional alcoholic beverages such as:

- "Is it the beverage made of purely barley or may it contains maize?''
- Why they selectively prefer to drink beverages prepared from barley and dislike that of maize or wheat?
- Is it may be due to the low alcoholic content of beverages made of barley or may beverages prepared from maize or wheat contain high level of methanol?

- Why most of the time people who consumed beverages made of maize are exposed to vomiting or high headache that may last for three or more days?
- The distilled beverage (arekie) produced from maize and/or wheat is very strong to drink and difficult to drink more compared to the arekie made of barley. Why is this so?, This study was designed because no scientific research have been conducted up to now on alcohol level comparison between widely consumed tella and arekie beverages prepared from different cereal grains separately.

1.3 Objectives of the study

1.3.1 General objective

To determine and compare ethanol and methanol content in traditional alcoholic beverages produced from different cereals.

1.3.2. Specific objectives

- To determine the ethanol content in traditionally prepared Ethiopian tella brewed from barley, maize and mixture of the two and tella purchased from selected vending houses
- To compare the ethanol level in the above mentioned tella beverages.
- To determine the methanol content in tella beverages produced from barley, maize and their mixture and the one purchased from vending houses and compare the level of methanol in these tella beverages.
- To determine the ethanol and methanol level in traditionally distilled Ethiopian liquor named arekie collected from vending houses and compare with the literature reviewed.

1.4 Significance of the Study

Alcoholic beverages are consumed by many people and hence their effects on people in terms of health, income and social status are quite significant. The ingestion of as little as 10 mL of methanol can cause permanent blindness and 30 mL can be fatal (Lal et al. 2001). The production and consumption of alcoholic beverages is likely to increase in the very near future, hence in order to protect the consumer the control of product quality become very necessary, through the application of acceptable sampling and testing procedures for the evaluation of the various inherent characteristics (DEAS, 2013). People also know harm caused by alcohol consumption and strategies to reduce the harmful use of alcohol endorsed by international as well as regional discussions which include; control on availability, law enforcement, regulating drink-driving, raising public awareness and counseling, treatment and rehabilitation (EAC, 2009). The Ethiopian public health authorities introduce control on vending houses by examining regularly the qualities of the drinks and hygiene practices to ensuring safe consumption of the beverages. Consumers will then be able to brew or identify their beverage from the type of the cereal with successful efforts in obtaining the greatest of health beneficial. Therefore, from this study after determining the different types of alcohols in different cereals' beverages and their alcohols content compared alcohol consumers will identify the cereal beverage with the lowest alcoholic content and also identify which cereal beverages contain methanol which is risk for their health.

2. LITERATURE REVIEW

2.1 History of Traditional Beverages

The history of alcohol in the ancient world dates back to before recorded time. Although no one knows when beverage alcohol was first used, it was presumably the result of accident that occurred at least ten thousands of years ago. According to the report of Olandeinde, 2002, however, alcoholic beverages have been used since the landing of pilgrims. In the developing world, fermentation is one of the oldest technologies used for food processing and preservation. On this technology depend up millions of people for preserving and often enhancing organoleptic and nutritional qualities of their food at costs available to the average consumer (Gotcheva et al., 2001; Tamang et al., 2005; Kalui et al., 2010). In some African countries in particular, cereals are used to produce indigenous fermented foods, non-alcoholic and alcoholic beverages. The oldest alcoholic drinks were fermented beverages of relatively low alcoholic contents (Rong, 2010). The early men probably used fermented beverage as a substitute for safe water (free from pathogens). Such beverages are usually non-intoxicating if consumed during the early stage of fermentation, as the alcohol level will still be low (Rong, 2010). Alcoholic beverages are consumed by many people and hence their effects on people in terms of health, income and social status are quite significant. Different types of alcoholic beverages were prepared in various parts of the world depending on the availability of diverse cereal crops. Their preparation was at a small scale and was only meant for domestic consumption. The technology that was employed for their production was primitive and archaic (Bhalla, 2009). In Africa, fermented alcoholic beverages are consumed in different occasions such as marriage, naming and rain making ceremonies (Zvauya *et al*, 1997), at festivals and social gatherings, at burial ceremonies and settling disputes. These include Egyptian bouza , Tanzanian wanzuki, Gongo, tembomnazi and gara Nigerian palm-wine, Kenyan muratna and uragela, and South African kaffir beer (Ashenafi et al ., 2001). These drinks are very popular, perhaps the most widely

consumed beverage types because of the nutritional, therapeutic, social and religious values attached to them (Solange, 2014). Ethiopia is one of the countries where a wide variety of traditional fermented beverages are prepared and consumed. The various traditional fermented beverages consumed in Ethiopia consist of both high alcoholic and low alcoholic beers (Getachew, 2015). Traditional recipes (ingredients) are handed down through generation and are still used for food processing in many developing countries (Kebede *et al*, 2002). The traditionally fermented beverages are low-cost product in all aspect as they are usually manufactured using only rudimentary equipment. Because of their cheapness, low income groups mostly consume them. Thus their handling and consumption often takes place under conditions of poor hygiene (Steinkraus, 1983). The lack of proper fermentation and distillation techniques by traditional or artisanal distilleries and some commercial operators may be a factor to high risk of methanol content in the products (Elijah 2016). As impurities and adulterations are the cases in many African countries, there are some complaints from regular customers in the study cities that some vending houses use certain plant roots and cement to increase the alcoholic potency of beverages (Yohannes et al., 2013). The other concerning aspect in many African countries is the rapidly changing drinking culture. In African society drinking alcohol has been an occasional and communal activity, associated with particular communal festivals (Room, 2014; Room et al., 2002). People do not drink alone or for the sake of drinking only, but these days this values are changing due to expansions of urbanization together with income inequality, along with the tendency to drink excessively inexpensive traditional and/or homemade high alcoholic beverages to alleviate their stress and have good time. According to the latest WHO (2014) global status report on alcohol consumption showed that about 30% of all alcohol consumed globally is unrecorded. This rate, however, is much higher in Eastern Mediterranean, South East Asia and Africa (56, 69 and 31%) respectively. Ethiopia is a country rich in cultural diversity. The variety of foods and beverages processed and consumed among the various ethnic groups are manifestations of this diversity. Ethiopia is one of the

countries where a wide variety of traditional fermented beverages are prepared and consumed. Among Ethiopian fermented beverages are varieties of Tella, Tej, Borde, Arekie, Keribo, and Korefe consumed in Ethiopia. Of traditionally fermented beverages in Ethiopia, the most popular alcoholic beverages are Tej (honey wine), tella (a malt beverage like beer) and Arekie (distilled liquor). These drinks are widely served on festive occasions and at social gatherings. The WHO global status report on alcohol and health released in 2014 showed that in Ethiopia the volume of unrecorded alcohol consumption is estimated to be 3.5 L of pure alcohol per capita indicating the highest consumption rate compared to other African countries such as Nigeria (1 L),Uganda(1.5 L) and Angola (1.6 L) (WHO, 2014). Consumption of alcohols from different brewing products possess several damaging effects such as, mental problems, job trouble, loss of control, impaired judgment in humans and irresponsible behaviors (Coleman and Cater, 2005; Bhargav *et al*., 2008;Sui *et al*., 2009). Alcohol stimulates insulin production, which speeds up glucose metabolism and can result in low blood sugar, causing irritability and possibly death for diabetics. Depending on the dose and the regularity of its consumption, alcohol is a more problematic causing heart disease, cancer, diabetes, acute respiratory failure or death (Guidot and Hart, 2005; Gun *et al*., 2006). There are considerable evidences in some African countries that produced alcoholic drinks are known to have toxic components (Reilly, 1976; Sanni, 1993). It is assumed that most traditional alcohol manufacturers are ignorant about the basic chemical compositions of the alcohol they produce and hence may end up producing alcoholic drinks with higher methanol content and other potential health threatening components (Jurgen, et al., 2014). Evidence supports a weaker, but possibly causal, relation between alcoholic beverages consumption and increased risk of cancers of the liver and breast (Lognecker, 1994). As well as potentially affecting the physical and mental health of individuals in many ways, chronic and heavy alcohol use can increase the risk of death (Rehm,et al; 2010), either directly, such as through acute alcohol poisoning or because alcohol causes a fatal disease such as cancer (Baan,et al; 2007**).** In addition to being produced in ethanol metabolism, acetaldehyde occurs naturally in alcoholic beverages. Limited

epidemiological evidence points to acetaldehyde as an independent risk factor for cancer during alcohol consumption, in addition to the effects of ethanol. This study aims to estimate human exposure to acetaldehyde from alcoholic beverages and provide a quantitative risk assessment (Lachenmeier, 2009).

Fusel oil is a collective name of isopentyl alcohol, 2-methyl-1-butanol, isobutyl alcohol, propyl alcohol, esters and aldehydes. It is toxic and has been shown to cause cancer in experimental animals (Fite et al., 2004). The toxicity of fusel oil is variable depending on the molecular weight of individual fusel oil components. These alcohols are responsible for the severe headache and thirst associated with hangover and also account for taste and flavor of alcoholic drinks (Fite et al., 1991). Various works have been reported in fermented food analysis, particularly alcoholic beverages in different parts of Ethiopia including the data with regards to methanol, fusel oil and ethanol contents of tella, areki and tej (Urga *et al*., 1997; Bekele *et al.,* 2001). The ethanol level, pH and sensory evaluation of tej, areki and tella were also reported by (Yohannes *et al*, 2013) that is significant variations in pH values of the tella within the sampling sites and pH value ranged from 4.00 to 4.99. There were also significant variations in the alcohol content of tella samples observed where the values ranged from 3.98 to 6.48 (% v/v) and averaged 5.17(%v/v). Three samples of the local fresh tella were taken and their alcoholic content was found as 3.04% (v/v) but in the second and third stages it was increased to the values of 3.35 and 3.75% (v/v) respectively (Berihu, et al, 2015).Tella is beer, which is normally 6% to 7% ABV (Abbink, 2002). The traditional beers had ethanol concentrations ranging from 2% v/v to 8% v/v. Most had concentrations of above 4% v/v, and the local distilled spirits had the highest concentration, up to 55% v/v (Joel, et al, 20170. Akpeteshie seemed to contain 40–45%Vol of ethanol implying that at least 400 ml of ethanol is contained in 1 L of the drink and hence may cause higher health risks when consumed excessively or without care (Kofi, 2017). Desta, in his survey of alcoholic content of some traditional beverage of Ethiopia, reported that the ethanol content of tella to range from 5.56 to 6.65% (v/v), and that of tej from 13.18 to 13.73% (v/v). The report also indicated that the alcoholic content of terra-areki and dagim areki to range from 30.56 –

36.83% (v/v) and 45.72 - 47.95% (v/v), respectively (Desta, 1977). The methanol content was reported in the ppm level; areki (320.87 ppm), tella (32.37ppm), and tej (45.67 ppm) (Fite et al., 2004).However, no methanol and higher alcohols content in the samples of tella, and areki was detected (Gizaw, 2006).Homemade distillation will, almost every time, lead to a methanol content which is higher than the one accepted in the EU. The average alcoholic content of the sample of tella is 6.36% (v/v) with a range of 5.21 - 8.03, where as that of the alcoholic content of terra-areki varies from 30.50 - 39.80% (v/v) with the average value of 37.22% (v/v) (Gizaw, 2006). Wide variations in results were obtained and this might be attributed to different methods of preparation of the beverages at the domestic level and the constituent variations are also suggested in literatures (Vogel and Gobezie, 1983). Conditions such as temperature, aeration and actions of the microorganisms also obviously affect the level of the alcohol in the samples (Sahle and Gashe, 1991).

2.2 Preparation of Ethiopian Traditional Alcoholic Beverages

2.2.1 Tella

Tella is one of the Ethiopian traditional beverages, which is prepared from different ingredients. Tella has various vernaculars in the various regions and is a malt beverage based on substrates such as barley, wheat. maize, millet, sorghum, teff or other cereals. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia (Ashenafi, 2006). Tella is widely brewed and consumed in both rural and urban part of Ethiopia. Tella is called Ethiopian traditional beer. Its production process is similar to beer making in that the grain starch is converted into sugars by malting. However, there is no yeast inoculation stage for fermentation but it utilizes the natural yeast present on the cereals. The dominant microorganism after the end of the first stage until the completion of tella fermentation was reported to be Saccharomyces cerevisiae and Lactobacillus pastorianumi (Sahle and Gashe, 1991). With regard to the substrate, there is no as such

basic difference between tella and beer. The bitterness of tella is from gesho, while that of beer is from hops ([Berhanu, 2014). Leaves of gesho are similar to hops in terms of its role in brewing; giving a bitter flavor and antibacterial effects for the shelf-life (Berhanu, 2014). In Korea, makgeolli is made with rice and nuruk which is a fermented whole wheat flour containing fungi, yeasts, and lactic acid bacteria. The pH value and alcohol content were 3.4 to 4.5 and 15 to 18% after 6 days' fermentation respectively
(Kim et al,2013). The alcoholic level of filter tella is between 3.5 to 6.48% (v/v) and pH value ranged from 4.00 to 4.99 (Yohannes et al.2013). For tella considered to be a good quality, the final ethanol content is in the range of 2-8% (v/v) and pH is 4-5 (Sahle and Gashe, 1991). How the tella is produced differs among ethnic groups, and their tradition and economic situation affect the kind of cereals they utilize. However, the basic processing steps are similar. Sahle and Gashe (1991) described the processes and microbiology of tella fermentation. The fermentation is divided into four phases. During the first phase, powdered leaves of gesho are mixed with water in a small earthen pot and allowed to ferment for four days. The fermenting material is commonly called tinsis. This is transferred to a large earthen pot and the second stage begins by mixing it with barley malt, pounded stems of gesho, pieces of kita and water. This is left to ferment for two more days. During the third stage, chopped pounded stems of gesho, bikil, enkuro and water are added to the container and the contents are mixed into thick slurry called difdif. This is also allowed to ferment for two more days. At the final stage, the container is filled with water to the brim and the contents are again mixed thoroughly. The container is then sealed to create anaerobic conditions and left to ferment for two more days. At the end of the fermentation, most suspended materials settle to the bottom of the container. The clear liquid is tella. Sometimes, at the end of the third stage, a smaller volume of water is mixed with the difdif and a more concentrated tella is obtained by filtering the difdif through a cotton cloth and keeping it in a closed container. Such tella is known as filter tella. Tella can be kept for 10-12 days. High quality tella is made with a relatively small quantity of water (WHO; 2014). The extent of baking or roasting determines the color of tella to be from light yellow to dark brown. Sahle and Gashe (1991) reported that

the first phase was important to extract the components of gesho. The liquid at this stage was very dark in color with a strong bitter taste. The microbial count increased markedly towards the end of the phase and reduction in content of total carbohydrate and reducing sugar occurred. The microbial flora consisted of molds, Lactobacillus spp. and other bacteria. Molds disappeared, however, towards the end of the phase. Ingredients added in the subsequent phases served as sources of fermenting microorganisms and increased amounts of carbohydrates and reducing sugars. Active fermentation resulted in vigorous foaming and bubbling. The final alcohol content of tella is 2 to 4%, while that of the filtered drink is 5 to 6%v/v (Mooha et al, 2015).

2. 2.2 Arekie

Arekie is a distilled beverage. It is a colorless, clear, traditional alcoholic beverage which is distilled from fermentation products prepared in almost the same way as tella except that the fermentation mass in this case is more concentrated (Fite et al., 2004). Arekie is distilled from a fermentation product known as yereki-tinsis. According to the report of Desta, 2001, yereki-tinsis is prepared by mixing powdered gesho leaves and powdered bikil with water to give a mixture of free flowing consistency, and which will be put aside to ferment for about five days. An amount of cereal (barley, wheat, maize or mixture of them) roughly equivalent to four times that of the bikil, is powdered, kneaded with water to make dough and baked into cakes. The hot cakes are broken into pieces, added to the first mixture and with more water, well mixed and again left aside to ferment for about four days. Portions of the second mixture are transferred to the traditional distillation apparatus and distilled to give what is known as terra-arekie. Arekie is usually brewed in rural and semi-urban areas and is used more commonly by farmers and semi-urban dwellers than by people who live in the cities. In cities, those who drink arekie are predominantly lower class people or those who have become dependent on alcohol and cannot afford to buy industrially produced alcohol (WHO, 2004). The alcoholic content of terra-arekie varies from 30.50 - 39.80% (v/v) with the

average value of 37.22% (v/v) and that of dagim arekie is 48%v/v (Gizaw, 2006). The highest value of ethanol in Dagim-arekie was reported to be 47.59% (v/v) with a mean value and range of 46.60 and 45.72 – 47.59% (v/v) respectively (Desta, 1977). Dagim-arekie is a stronger type of terra-arekie, which is prepared in the same way as terra-arekie, except that the distillation process is allowed to proceed for a shorter period of time, or three volumes of terra-areki are redistilled to give about one volume of dagim-arekie (Desta, 1977). Since the government has no control over the production of locally brewed alcoholic drinks, it is difficult to estimate the amount of alcohol production and consumption in Ethiopia. However, the unrecorded alcohol consumption is estimated to be 1.0-liter pure alcohol per capita for population older than 15 years of age for the years after 1995 (WHO, 2004).

2.2.3 Tej

Tej is a home-processed, but also commercially available honey wine. It is a beverage mainly used for great feasts, such as weddings and the breaking of fasting. It is prepared from honey, water and leaves of gesho (Rhamnus prenoides). Sometimes, widely for commercial purposes, mixture of honey and sugar could be used for its preparation. In cases where sugar is used as part of the substrate, natural food coloring is added so that the beverage attains a yellow color similar to that made from honey (Fite *et al*., 1991). According to Vogel and Gobezie, (1983), during the preparation of tej, the fermentation pot is seasoned by smoking over smoldering gesho 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 and put back to the fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15 -20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and gesho. Good quality tej is yellow, sweet, effervescent (contains bubbles of gas) and cloudy due to the content of

yeasts. The flavor of tej depends upon the part of the country where the bees have collected the nectar and the climate.

2.2.4. Shameta

Shameta is a widely consumed beverage in different regions of Ethiopia, which is low in alcohol content, made by overnight fermentation of mainly roasted barley flour and, consumed as meal-replacement (Bacha *et al*., 1999). Ready to consume Shameta has a high microbial count made up of mostly lactic acid bacteria and yeast (Bacha et al., 1999). These microorganisms make the product a good source of microbial protein. However, Shameta has poor keeping quality because of these high numbers of live microorganisms and becomes too sour about four hours after being ready for consumption (Mogessie, and Tetemke, 1995).

2.2.5. Borde

Borde is a traditional fermented beverage of Ethiopia, a common meal replacement in Southern Ethiopia, and some other parts of the country (Mogessie and Tetemke, 1995). Borde is prepared from unmalted maize, barley, wheat, finger millet, sorghum and/or tef and their malt, except sorghum and tef (Kebede *et al*., 2002). Maize, followed by barley and wheat, was found to be the most common ingredient of borde both as malt and un malted ingredient in southern Ethiopia, whereas wheat has been reported to be the preferred unmalted ingredient in Addis Ababa (Bacha, 1997). Borde is also used for medical and ritual purposes. Consumers believe that borde enhances lactation and mothers are encouraged to drink substantial amounts of it after giving birth (Kebede et al., 2002). Borde is also considered to alleviate malaria, diarrhea, constipation and abscesses. Garlic, fresh chili, ginger and salt are offered as appetizing accompaniments to reduce the feeling of fullness and encourage the intake, which may also contribute to some medical effects, if any (Kebede et al., 2002). Borde has a short shelf life as it turns too sour to consume after about 4 hours after completion of phase IV of the fermentation. It is, nevertheless, one of the important nutritious and low

alcohol beverages in Ethiopia. Borde is one of the various nutritious and low alcoholic traditional fermented beverages in Ethiopia. The scaling up of such products, although important, may have to be undertaken with great care so as not to lose the nutritive value as well as the public acceptance of the beverages. Identification of the strains important for fermentation and optimization of the process parameters should be done in detail to design mechanisms for production of industrial-based products. Kebede Abegaz et al. (2004), for example, studied effect of technological modification on fermentation of borde and suggested simpler and shorter process that can yield acceptable borde, but the microbial safety of the product was questionable.

2.2.6 Keribo

Keribo is an indigenous traditional fermented beverage produced and consumed in different parts of the country, including Jimma zone. It is produced mainly from barley and sugar. Fermented Keribo constitutes a major part of the beverages being served on holidays, wedding ceremony and also as sources of income of many households in Jimma zone. The popularity of this traditional fermented beverage is more reflected among the religious groups and those do not like alcoholic drinks. Being considered as a non- or low- alcoholic beverage, Keribo is popular among both adults and children. It has poor keeping quality with shelf-life of not more than a day or two and it has a pronounced characteristic of the deteriorating beverage at the end of 48 h of fermentation (Getachew, 2015). Keribo is a traditional, non-alcoholic, dark brown colored fermented beverage commonly consumed in rural and urban areas of Jimma zone, southwestern of Ethiopia, with some similarity to Boza of Bulgaria, Albania, Turkey and Romania (Blandino et al., 2003). It is produced by an over-night fermentation of cereal (barley) predominantly by activities of LAB like the fermentation of shamita (Bacha et al., 1999). the addition of sugar and yeast to un-malted, deeply roasted and boiled barley to initiate fermentation of Keribo is the possible source of LAB responsible for Keribo fermentation (Rashid et al.,2013).

3. MATERIALS AND METHODS

3.1 Sampling and Sample Preparation

In this study different home brewed tella samples from different cereals and purchased tella and areki samples from vending houses were considered for chemical analysis. Three pure barley tella, three pure maize tella, and three barley and maize mixture tella types were prepared at home under the same conditions with equal amount of ingredients at the same time intervals. Three tella samples were bought from three different vending houses selected purposely where most people consume regularly. Three Arekie samples were also bought from three different vending houses as tella samples. Finally the three, three beverage samples of each cereal type were mixed in equal amount to obtain a bulk sample of barley tella, maize tella, mixture of barley and maize tella, purchased tella and purchased areki samples. This was done because instead of analyzing separately, analyzing of the mixture reduces the variance and resource consumption and triplicate analysis was performed for each of the samples. Therefore, a total of four tella samples and one areki sample were considered for analysis. Each sample was mixed in one liter plastic bottles and screw-capped and were kept in a Refrigerator at a temperature of 4^{o}C until chemical analysis. The four tella samples were analyzed using the Alcolyzer beer method and the arekie sample was analyzed by pycnometer method in the laboratory of Conformity Assessment Enterprise (ECAE) at Addis Ababa. The other four liters of tella samples and one liter of arekie sample were first distilled using Clevenger distillation apparatus and dried by salting out effect in the laboratory of Adama Science and Technology University (ASTU) at Adama. The distillates were then placed in a refrigerator at a temperature of 4^{o}C until chemical analysis using GC method. These prepared through distillation were used for the determination of methanol content and some higher alcohols in the beverages. At Adami Tulu laboratory solvent and internal standard added to each sample and to standard methanol for extraction before injection in to GC column.

3.2 Experimental

3.2.1 Materials and apparatus

The materials and Laboratory apparatus used throughout the experiment include: 500 mL round bottom flask, measuring cylinder, 50-250 ^{o}C Thermometer, pipettes, 25-50 mL pycnometer, 100 mL volumetric flasks, analytical balances, gas chromatographic unit and Clevenger apparatus.

3.2.2 Chemicals and reagents

Analytical grade reagents and distilled water were used in all tests. Ethanol and methanol standards (purity 99.9%), Na_2SO_4, Carrier gas (N_2), ethyl acetate (internal standard), chloroform-solvent, tella and arekie samples were used during the experiment.

3.2.3. Determination of Ethanol Level of the Samples

3.2.3.1 Determination of ethanol level of tella

The ethanol level of tella and arekie samples was determined in the laboratory of Ethiopian Conformity Assessment Enterprise (ECAE), Addis Ababa. The ethanol level of tella samples was determined by Alcolyzer beer method. Alcolyzer beer p/n 88387 DMA 5000M, Anton Paar GmbH-Austria, is suitable for research work due to its even higher accuracy. Adjustment/calibration, sample preparation and operation are simple. Measuring times are short and results are highly accurate. The system measures all types of beers, including non-alcoholic beers, beer mixtures, RTD (ready to drink) alcoholic beverages, and also cider and molasses (bioethanol). The whole system is operated via a touchscreen on the DMA M master instrument. The Alcolyzer Beer Analyzing System determines the alcohol content and further parameters such as

density, original extract, and real extract, degree of fermentation, pH and turbidity - all in one measuring cycle, all from one single sample. Measuring range: alcohol content from 0 %v/v to 12 %v/v, Original extract from 0 °Plato to 30 °Plato, Density from 0 g/cm^3 to 3 g/cm^3, pH from 0 pH to 14 pH. Repeatability (standard deviation (stdev.)) of Alcohol content, 0.01 %v/v, Original extract: 0.03 °Plato, Extract content: 0.01 %w/w, Density: 0.000001 g/cm3 (DMA 5000 M), pH: 0.02 pH (in the range 3 pH to 7 pH). Due to its selectivity and linearity, the measuring method only requires adjustment (calibration) with water and an alcohol/water solution. Highly accurate measurement with no influence from other sample ingredients.

Tella sample analysis

Prior to any measurement the tella samples were filtered with fluted filter paper in to a 30mL plastic tubes and then taken to the circular system of the Alcolyzer beer system arranging them in triplicate in which distilled water was placed in between each triplicate tella samples for calibration, and then the laboratory technician operated the system by touchscreen after which the alcohol content and other parameters were observed as the results of the biochemical analysis of the samples on the screen window of the system from which one can read or printout the results.

3.2.3.2 Determination of ethanol level of arekie

The ethanol level of arekie sample was also determined in the laboratory of Ethiopian Conformity Assessment Enterprise (ECAE), Addis Ababa. The analysis was made by pycnometer analysis method (ES 819:2012). The pycnometer is a long necked, model: Duran-245, Germany. Density determination by pycnometer is a very precise method. It uses a working liquid with well-known density, such as water. The mass of an empty pycnometer in gram, mass of pycnometer with distillate sample in gram at 20 ^{o}C and mass of pycnometer with distilled water in gram at 20 ^{o}C were measured using

a balance(Sartorius BP211D),Germany. Then the relative density of the sample was calculated from mass of pycnometer with the sample, mass of pycnometer with distilled water and mass of empty pycnometer with the following expression:

$$R.D. = \frac{M3-M1}{M2-M1} \qquad \text{(Gizaw, 2006)}$$

Where, R.D =Relative density of distillate alcohol at 20 ^{o}C.

M_3 =Mass of pycnometer with distillate sample in gram at 20 ^{o}C.

M_2 =Mass of pycnometer with distilled water in gram at 20 ^{o}C.

M_1 =Mass of empty pycnometer in gram.

The alcohol content percent by volume (ABV%v/v) of the samples were then taken from beverage reference book ES 827.

3.2,4 Determination of Methanol

The determination of methanol level and some higher alcohols in the beverages was attempted by Gas chromatography (Model: Agilent 7890A GC system with auto sampler, HP-5 column, FID detector chem-staion software, US). Prior to GC analysis the samples were distilled using a Clevenger apparatus. To a round bottom flask, 100mL of the sample and 50mL distilled water were added and then distilled until the 100mL distillate was recovered. This was done for each of the five samples. The distillates were then collected and dried by salting out effect using Na_2SO_4 as dehydrating agent. These samples were then placed in the refrigerator at 4 ^{o}C in the ASTU laboratory until chemical analysis. The GC analysis for the determination of methanol was performed at Adami Tulu Eastern shoa pesticide processing laboratory. The samples were then

analyzed by GC with condition as follow: GC Model: Agilent7890A, GC Condition: Column type HP-5capillary column 5% phenyl methyl siloxane 325 ^{o}C:30 m x 320 µm x 0.25 µm , mode split, heater on: 200 ^{o}C, pressure on:11.761 psi, total flow on:186 mL/min, septum purge flow:3 mL/min, split ratio:60:1, split flow:180 mL/min, run time:21 min, Oven temperature Programmed 40 ^{o}C for 5min then10 ^{o}C/min to 150 ^{o}C for 5 min, Detector FID Temp: 300 ^{o}C, H_2 flow:70 mL/min, air flow:400 mL/min, makeup flow:7 mL/min, gas Pressure: 2.5 mL/min, flow 0.5 mL/min, flow program:3 mL/min for 5 min, syringe size:1 µL.Then with these conditions the determination of methanol was performed for each sample using chloroform as a solvent. The standard solution of methanol was prepared by mixing 0.951 gm methanol purity 99.9% with 10mL chloroform (solvent) to mimic the chemical composition of alcoholic beverages and was extracted exactly as the samples. Therefore, there was no need of recovery correction (Croitoru, et al, 2013).1 µL each of these prepared standard solution and analyte solution were injected into the GC column in separate run after which the chromatograms were observed. Based on the peak area of methanol in the standard, peak area of methanol in the analyte samples, weight of standard, weight of analyte taken and purity of standard solution, the methanol content by percentage in each analyte sample was calculated by the following formula:

$$\text{ABV \% methanol} = \left(\frac{\text{Area of methanol in sample}}{\text{Area of methanol in standard}}\right)\left(\frac{\text{(Weight of standard}}{\text{Weight of sample}}\right)\text{(purity of standard)}.$$

3.3 Data analysis methods

The two most important elements of a chromatographic test method are accuracy and precision. Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Precision measures of how close individual measurements are to each other (Joseph, 2010). Measures of accuracy and precision were calculated based on the data generated, given the glassware and equipment used, to

evaluate the skill of the user as well as the reliability of the instrument and glassware. In order to compare the means of ethanol content, one-way ANOVA (significance level p = 0.05) was performed on spss software.

Accuracy of Alcolyzer beer method can be found in two ways:

The first one is using two methods comparison, Alcolyzer beer method and pycnometer method (ES318) (Table 1) and the second one is using true (actual) value (Table 2). To use both methods first 100%v/v alcohol was taken and diluted to 4%v/v alcohol by using the law of dilution: $c_1v_1 = c_2v_2$. This is because the alcolyzer beer meter could not measure alcohol content greater than 12%v/v. Then 4% v/v alcohol was taken as the true value and its alcohol content was measured using both alcolyzer beer meter and pycnometer. Then using the two methods comparison the accuracy of the method was calculated from their means using the following expression::

$$\text{Accuracy} = 100 - \frac{2(x1-x2)}{(x1+x2)} \times 100$$

Where:

X_1 = mean value of alcolyzer meter reading

X_2 = mean value of pycnometer reading

The expression: $\frac{2(x1-x2)}{(x1+x2)} \times 100$ indicates the percent error.

Table 1: percent accuracy of the instrument by two method comparison

Parameter	Nº of analysis	Method used		
		Alcolyzer meter	Pycnometer (ES318)	Accuracy
Relative density	A	0.99252	0.99226	99.97%
	B	0.99252	0.99228	
	C	0.99252	-	
	Mean(x)	0.99252(x_1)	0.99227(x_2)	
Alcohol content (%v/v)	A	3.96	3.97	99.62%
	B	3.99	4.04	
	C	3.96	-	
	Mean(x)	3.97(x_1)	4..00(x_2)	

The second one is accuracy using actual (true) value. In the science of measuring things, "accuracy" refers to the difference between a measurement taken by a measuring tool and an actual value (Banas, 2018). The formula is given as follows:

$$\text{Accuracy} = \left(\frac{measured\ value}{true\ value}\right) \text{x}100$$

Table 2: percentage accuracy in terms of trueness

Parameter	Nº of analysis	Alcolyzer meter reading	Accuracy
Relative Density	A	0.99252	100.01%
	B	0.99252	
	C	0.99252	
	Mean value(x)	0.99252	
	True value	0.99236	
Alcohol content (%/v)	A	3.96	99.25%
	B	3.99	
	C	3.96	
	Mean value(x)	3.97	
	True value	4.00	

4. RESULTS AND DISCUSSION

4.1 Chemical properties of the samples

4.1.1 pH values of tella samples

Table 3: pH values of the samples of traditional beverages (n=3, triplicate analysis)

sample	BTC	MTC	BMTC	PTC
pH	4.57 ± 0.02	4.55 ± 0.04	4.41 ± 0.007	5.43 ± 0.2

Where: BTC = barley tella composite, MTC = maize tella composite

BMTC = mixture of barley and maize tella composite, PTC =purchased tella composite

Table 1 shows the pH of the tella samples. The average pH values of tella samples ranged from 4.41 to 5.43. It is noted that the home brewed tella samples were more acidic than the purchased tella sample. Statistical one way ANOVA analysis indicated that there is significant variation between the brewed tella samples and the purchased one, however, no significance difference was observed in pH between BTC and MTC, MTC and BMTC at P = 0.05(appendix-1). These wide variations in results were attributed to different methods of preparation of the beverages at the domestic level and the constituent variations are suggested in literatures (Vogel and Gobezie, 1983). This might be due to aging, fermentation steps and conditions of temperature, aeration and hygiene of the apparatus (utensils) used (Fite, et al., 2004).

4.1.2 The Ethanol Content and specific gravity of tella Samples

Table 4: percent ethanol content, specific gravity and pH of traditional tella

Sample	BTC	MTC	BMTC	PTC	Means
Specific gravity	1.0016 ± 0.0	0.9992 ± 0.0	1.0019± 0.0	1.0039± 0.0	1.0016
ABW%w/w	5.13 ± 0.02	5.46 ± 0.03	4.57 ± 0.02	2.36 ± 0.04	4.38
ABV%v/v	6.51 ± 0.02	6.91 ± 0.04	5.80 ± 0.03	3.00 ± 0.04	5.56
pH	4.57 ± 0.02	4.55 ± 0.04	4.41 ± 0.007	5.43 ± 0.2	4.74

Table 5: Ethanol content of home prepared Tella Beverages (n=3)

Sample	BTC	MTC	BMTC	Means
Specific gravity	1.0016 ± 0.0	0.9992 ± 0.0	1.0019 ± 0.0	1.0009 ± 0.00
ABW%w/w	5.13 ± 0.02	5.46 ± 0.03	4.57 ± 0.02	5.05 ± 0.02
ABV%v/v	6.51 ± 0.02	6.91 ± 0.04	5.80 ± 0.03	6.41 ± 0.03
pH	4.57 ± 0.02	4.55 ± 0.04	4.41 ± 0.007	4.51 ± 0.02

he data on the above table should follow the explanation that the average alcoholic content of the samples of tella is 5.56%v/v with the range 3.00 to 6.91(Table 4). The average alcoholic content of the samples of tella brewed at home is 6.41 with the range 5.80 to 6.91(Table 3). There is significance variations in ethanol content between each of the tella samples prepared from different cereals under the same conditions of temperature and pressure, at the same time interval and the one bought from vending houses as indicated by statistical one way ANOVA analysis at p= 0.05 level (appendix-2). This variation might be due to the differences in the type of raw materials (ingredients) used, methods of

preparations and aging. Conditions such as temperature, aeration and actions of the microorganisms also obviously affect the level of the alcohol content in the samples (Sahle and Gashe,2001). As indicated in (Table 2) the average ethanol content of maize tella (6.91%v/v) > barely tella (6.51%v/v) > the mixture of barley and maize tella beverage (5.80%v/v) > purchased tella (3.0%v/v). The variation in ethanol content between barley tella and maize tella beverages is as expected whereas the higher in ethanol level of barley tella than that of tella prepared from the mixture of barley and maize is unexpected result. The mean alcoholic content of Ethiopian traditional beverage varies from report to report (Desta, 2001). Desta, in his survey of alcoholic content of some traditional beverages of Ethiopia, had reported that the ethanol content of tella to range from 5.56 to 6.65% (v/v) (Desta, 2001). Fite et al, had reported 6.57%v/v as a mean ethanol content of tella samples collected from eight different parts (Bichena, Bahar Dar, Dangla, Emmanuel, Finote Selam, Debre Berhan, Ataye, and Addis Ababa) of Ethiopia. Gizaw had reported that the mean alcohol content of tella found to be 6.36% (v/v) with a range of 5.21 - 8.03(Gizaw, 2006). The alcoholic level of tella is between 3.5 to 6.48% (v/v) (Yohannes, et, al., 2013). Sahle and Gashe reported that a good quality of tella consists the final ethanol content in the range of 2-8% (v/v) (Sahle and Gashe, 2001). In comparison, the mean alcoholic content of tella,5.56%v/v, in this study is in the range to that of the above reported beverages by different researchers. However, there is a wide variation of home brewed tella beverages with some reported alcoholic beverages such as tella collected from Debre Berhan, Ataye and Addis Ababa had alcohol content of 2.4-3.3%, 2.1-2.7% and 1.6-2.8%, respectively (Fite et al., 2004).But the tella samples collected from vending houses in Asella town in this study are in comparison with the average ethanol content of(3.0 %v /v). According to the standard of European brewery convention (EBC) a good quality of industrial brewery have a range from 4.5 to 5.3% (v/v) ethanol content (Berihu, et al., 2015). Thus, the ethanol content of the tella beverages considered in this study is almost nearer to European standard but not in the range.

4.1.3 Arekie sample analysis

Table 6: Ethanol content of arekie beverage (n=2)

Sample	M1	M2	M3	ABV%v/v
PAC_1	61.5074	161.3478	154.7027	49.09
PAC_2	56.8250	156.4714	149.8720	48.94
Mean	59.1662 ± 3.31	158.9096 ± 3.45	152.2874 ± 3.42	49.02 ± 0.11

The average ethanol content of arekie liquor considered in this study is 49.02(Table 4) is more concentrated compared with the reported values (37.22) for terra-arekie and even higher than that of dagim arekie (48.00) (Gizaw, 2006). The report indicated that the alcoholic content of terra-areki and dagim-areki to range from 30.56 - 36.83% (v/v) and 45.72 - 47.95% (v/v), respectively. The other report was that of (Teshome et al.,2017) areki drinks contained an average alcoholic strength of 47.56%v/v which is higher and even much higher than the maximum allowable limits for similar spirit drinks in Europe (Regulation, 1989). Hence in comparison of the ethanol content of the arekie beverages reported above by different researchers, the average ethanol content of arekie considered in this study 49.02 is very high. High alcoholic drinks such as areki have a great potential to cause detrimental health effects through the huge increment of alcoholism (Teshome et al., 2017). The cost of alcoholism to the society in terms of crimes, accidents, medical and custodial care, broken homes, wasted lives and human misery is beyond calculation

7: Table Methanol content of tella beverages and arekie

sample	Amount of sample in gm	Amount of methanol in std.	Purity of methanol	Peak area of methanol in std	Peak area of methanol in sample	%v/v of methanol in sample
BTC	0.9454	0.951	99.9	10885	241.378	2.228
MTC	0.9485	0.951	99.9	10885	10.474	0.0964
BMTC	0.998	0.951	99.9	10885	3.581	0.031
PTC	0.9658	0.951	99.9	10885	21.42	0.194
PAC	0.9929	0.951	99.9	10885	5.374	0.0677

The GC analysis of the traditional tella beverages both home prepared and that bought from vending houses and also arekie liquor indicates that there is methanol in all of the samples considered for chemical analysis in this study (Table 7).This is somewhat out of expectation. Because as found in many reviews the methanol alcohol might not be found in beverages made of cereals or found in very small quantity that is in parts per million [Gizaw, 2006]. The other unexpected result obtained from the GC analysis is that the methanol content in barley tella is higher than that of maize tella. But it was expected that the reverse is true. This is because the headache observed on many maize tella consumers was due to the presence of much amount of methanol. However, the result of this study reveals that there may be other compounds which can be the cause of the effect. This implies that there is a need of further investigation on the traditional alcoholic beverages and liquors.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The study indicated that the alcoholic content and pH values both of home brewed traditional tella beverages and the one bought from vending houses vary considerably as indicated by statistical one way ANOVA ($p < 0.05$). This variability of local beverages could be attributed to the spontaneous fermentation**,** different methods of preparation, aging, type of raw materials used, conditions such as temperature, aeration and actions of the microorganisms that obviously affect the level of the alcoholic content and pH values in the samples. Clear understanding of the procedures involved in the process of making of the traditional beverages could help to design mechanism for production of an industrially based finished product. It was expected that methanol may not be found or found in very small amount, that is in parts per million as reviewed in different literatures in cereal based alcoholic beverages, however, this study indicated that there is methanol in all samples under study even if its level is different in each of the beverage in which the level in barley tella beverage is the highest (unexpected) and in the mixture of barley and maize tella beverage is the least. This might be due to contamination of barley tella either during fermentation process or in the raw materials, during sample preparation or during chemical analysis.

5.2 Recommendation

1. This study indicated that there are significant variations in alcohols content and pH values in the tella samples. Thus, it requires further investigations for the causes of these variations between tella beverages.

2. Not only the variation in ethanol content and pH values in the samples but also methanol alcohol which is risk for health is also found in all of the beverages under study and many other compounds as the GC analysis indicated. Therefore, it requires further study which is left for the next researcher with the latest GCMS instrument.

3. From the point of view of public health, investigation on the mechanism of production and means to avoid predominance of harmful contents are necessary.

4. In general control in the production and supervision with the development of comprehensive national alcohol policy is recommended.

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6. REFERENCES

Abbink, J. (2002). Drinking, prestige and power: Alcohol and cultural hegemon in Maji, southern Ethiopia. In D. F. Bryceson (Ed.), Alcohol in Africa: Mixing business pleasure and politics (pp. 161–178). Portsmouth, NH: Heinemann.

Abegaz, K., Beyene, F., Langsrud, and Narvhus, JA. (2002) Indigenous Processing methods and raw materials of borde, an Ethiopian traditional Fermented beverage. J .Food Technol Africa , 7, 59–64.

Ashenafi M. A. (2006): Review on microbiology of indigenous fermented foods and beverage Ethiopia. Ethiop J Biol Sci; 5:189 to 245n traditional alcoholic beverages Afr J Food Sci, 7, 399–403

Baan, R., Straif, K., Grosse, Y., Secretan, B., Ghissassi, F. E., Bouvard, V., et al. (2007). Carcinogenicity of alcoholic beverages. The Lancet Oncology, 8(4), 292–293.

Bacha K, Mehari, T. and Ashenafi, M., (1999): Microbiology of the fermentation of Shamita, a traditional Ethiopian fermented beverage. SINET. Ethiop J Sci.,22, 113- 126.

Bacha, K., (1997): Microbial ecology of borde and shamita fermentation. M.Sc. Thesis, Department of Biology, Addis Ababa University, Ethiopia.

Bekele, B., Tetemke, M., and Mogessie, A. (2001). Chemical and nutritional properties of 'tej', an indigenous Ethiopian honey wine: variations within and between production units. J. Food Technol. 6(3), 104-108.

Berhanu A., (2014). Microbial profile of tella and the role of gesho (Rhamnus prinoides) as bittering and antimicrobial agent in traditional tella (Beer) production. Int Food Res J. 21:357e65.

Berihu T., Getachew G., Tewodros A., and Harikrishna R. S.(2015). Determination of Alcoholic Content and Other Parameters of Local Alcoholic Beverage (Tella) at Different Stages in Gondar, Ethiopia: 4(6), 2319-4413

Bhalla T.C., Thaku N., Thaku A., Pratush A.(2009). Cereal based alcoholic beverages. In book: Fundamentals of Food Biotechnology, New Delhi. India.

Bhargav, S., Panda BP., Ali, M., and Javed S. (2008). Solid-state Fermentation : An Overview. Chem. Biochem. Eng. Quart. 22:pp. 49-70.

Blandino, A., Al-Aseeria, M.E., Pandiellaa, S.S., Canterob, D. Webba, C (2003). Review: Cereal-based fermented foods and beverages. Food Res. Int. 36: 527–543

Cole, V.C. and Noble, A.C. (2003) Flavor chemistry and assessment. In: Fermented Beverage Production, A. G. H Lea and J. R. Piggott, Eds., Kluwer/Plenum Publishers: New York, pp. 361-385

Coleman, L.M.and Cater, S.M. (2005). A qualitative study of the relationship between alcohol consumption and risky sex in adolescents. Arch. Sex. Behav. 34: pp. 649-661.

Croitoru MD, Topor Elena, Fülöp Ibolya, Fogarasi Erzsébet(2013). A Survey on the Methanol Content of Home Distilled Alcoholic Beverages in Transylvania (Romania), Acta Medica Marisiensis ;59(4):206-208

DEAS (2013): Alcoholic beverages — Methods of sampling and test

Desta Birhanu, (2001). A survey of the alcoholic contents of traditional beverages, Ethiop. Med. J., 15, 65-68.

EAC (2009) Alcohol an Obstacle to Development in East Africa. Proceedings of the East Africa Conference on Alcohol; pp, 13-14, Arusha: Tanzania.

EAC (2013) Alcoholic beverages — Methods of sampling and test.

Elijah, I., (2016). Methanol contamination in traditionally fermented alcoholic beverages: the microbial dimension. 5(1): 1607. doi: 10.1186/s40064-016-3303-1

Fite, A., Tadesse, A., Urga, K., and Seyoum, E. (2004). Methanol, fusel and ethanol contents of some Ethiopian traditional alcoholic Beverages.SINET.Ethiopia J. SCi.14,19- 27

Getachew T., (2015). A review on Traditional Fermented Beverages of Ethiopian. Journal of Natural Sciences Research, 5(15), 2224-3186

Gizaw, D. (2006): Determination of ethanol level in beverages. Master Thesis, Addis Ababa University, Addis Ababa Google Scholar.

Glithero NJ, Ramsden SJ, Wilson P (2013). Barriers and incentives to the production of bioethanol from cereal straw: a farm business perspective. ELSEVIER - Energy Policy, 161-171.

Guidot, D.M.and Hart, C.M. (2005). Alcohol abuse and acute lung injury: epidemiology and pathophysiology of a recently recognized association. J. Investig. Med.53, 235-245.

Gun, RT, Pratt N, Ryan P, Gordon I, Roder D. (2006). Tobacco and alcohol- related mortality in men: estimates from the Australian cohort of petroleum industry workers. Aust. N. Z. J. Public Health 30,318-324.

Holzapfel W (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. Int. J. Food Microbial. 75(3):197-212.

Holmes, Michael V.; et al. (2014). "Association between alcohol and cardiovascular disease: Mendelian randomization analysis based on individual participant data". BMJ. 349: g4164. doi:10.1136/bmj.g4164. PMC 4091648 Freely accessible. PMID 25011450.

Isaac-Lam, Meden F. (2016). Determination of Alcohol Content in Alcoholic Beverages Using 45 MHz Benchtop NMR Spectrometer. International Journal of Spectroscopy

Joel M. Francis , M.D.,Heiner Grosskurth , M.D., Saidi H. Kapiga , M.D., Helen A. Weiss, Joseph Mwashiuya (2017). Ethanol Concentration of Traditional Alcoholic Beverages in Northern Tanzania. Journal of Studies on Alcohol and Drugs, 78(3), 476–477

Joseph M. Betz,a Paula N. Brown,b and Mark C. Roman(2010). Accuracy, Precision, and Reliability of Chemical Measurements in Natural Products Research. 82(1): 44–52. doi: 10.1016/j.fitote.2010.09.011

Jurgen R, Shalini K, Elisabeth L, Maximilien XR, Andriy VS, Kevin DS, et al(2014). A systematic review of the epidemiology of unrecorded alcohol consumption and the chemical composition of unrecorded alcohol. Addiction. 880–93.

Kim E, Chang YH, Ko JY and Jeong J(2013). Physicochemical and microbial properties of the Korean traditional rice wine, makgeolli, supplemented with banana during fermentation. Prev Nutr Food Sci, 18:203e9.

Kofi, S., (2017). Determination of methanol and ethanol concentrations in local and foreign alcoholic drinks and food products (Banku, Ga kenkey, Fante kenkey and Hausa koko) in Ghana

Kruse, JA. (October 2012). "Methanol and ethylene glycol intoxication.". Critical care clinics. 28 (4): 661–711.

Lachenmeier DW.(2007).Rapid quality control of spirit drinks and beer using multivariate data analysis of Fourier transform infrared spectra. Food Chem.101:825–32.

Lachenmeier DW[1], Kanteres F, Rehm J.(2009). Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. 104(4):533-50. Doi

Lal J, Kumar CV, Suresh MV, Indira M, Vijayammal PL(2001). Effect of exposure to country liquor (toddy) during gestation on lipid metabolism in rats. Plant Foods Hum Nutr; 56:133–43.

Longnecker, M. P., (1994): Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. Cancer causes control 5 (1): 73 – 41.

Mooha Lee, Meron Regu , Semeneh Seleshe (2015). Uniqueness of Ethiopian traditional alcoholic beverage of plant origin, Tella, J. of Ethnic Foods. 2 (2015) 110-114

Mureithi, K. (2002,). Kenya's love of "poison." BBC News Online. Available: http://news.bbc.co.uk/1/hi/in_depth/ Africalive/2213006.stm.

Olandiende, F.O; Nwankwo, E.I; Moro kola, O.A; Amosu, M.A., and Farayola, B. (2002): Determination of indigenous and foreign alcoholic beverages' levels in urine by quantitative infrared spectroscopy, African Journal of Biomedical Research, 5(1-2), 73-76.

Pederson, S.C. (1979): Microbiology of Fermentation.2nd ed. AVI Publishing Co. Inc. West Port. Connecticut.

Rashid Abafita Abawari (2013). Microbiology of Keribo Fermentation: An Ethiopian Traditional Fermented Beverage. Pakistan Journal of Biological Sciences, 16: 1113-1121.

Rehm, J., Baliunas, D., Borges, G. L., Graham, K., Irving, H., Kehoe, T., et al. (2010). The relation between different dimensions of alcohol consumption and burden of disease: An overview. Addiction, 105(5), 817–843.

Rehm, J., Sulkowska, U., Manczuk, M., Boffeta, P., Powles, J., Popova, S., & Zatonski, V. (2007). Alcohol accounts for a high proportion of premature mortality in central and eastern Europe. International Journal of Epidemiology, 36, 458–467.

Rong, T. (2010). Chemistry and biochemistry of dietary polyphenols. Nutrients, 2, 1231-1246.

Room, R., Jernigan, D., Carlini MB, G., Ureje, O., Mäkelä ,K., Marshall, M., and Partanen, J. (2002). Alcohol and the developing world: a public health perspective: Finnish Foundation for Alcohol Studies in collaboration with World Health Organization.

Room, R. (2014). Alcohol control policies in low-and middle-income countries: Testing impacts and improving policymaking practice. Int. J. Alcohol Drug Res. 3(3), 184-186.

Room, R., Babor, T., & Rehm, J. (2005). Alcohol and public health. The Lancet, 365(9458), 519–530.

Sahle, A. and Gashe, B.A. (2001): The microbiology of tella fermentation. SINET Ethiop. J. Sci., 14, 81 - 92.

Sanni, A. (1993). The need for process optimization of African fermented foods and beverages. Int. J. Food Microbial. 18(2), 85-95.

Shale, K., Mukamugema, J., Lues, R., and Venter, P. (2014). Possible microbial and biochemical contaminants of an indigenous banana beer Urwagwa: A minireview. Afr. J. Food Sci. 8(7), 376-389.

Shewakena S,(2015). Studies on the levels of total polyphenol, flavonoid, tannin and antioxidant capacity of selected Ethiopian fermented traditional beverages. Master Thesis, Addis Ababa University, Addis Ababa Google Scholar.

Solange, et al., (2014). Review on African traditional cereal beverages, American J. of Research Communication, 2(5): 103-153

Spedding G.(2015). Alcohol Measurements: Chromatography (Gas Chromatography - GC and GC-Mass Spectroscopy, High Performance Liquid Chromatography - HPLC); Densitometry; Enzymatic and Spectroscopic (Near-Infrared - NIR and Nuclear Magnetic Resonance - NMR) Methods. BDAS, LLC WPSP#3,12-11.

Steinkraus, K.H. (1983): (Ed) Handbook of Indigenous Fermented Foods. Marcel Dekker, Inc. New York. 34

Steinkraus, K.H.(1986):Fermented foods, feeds and beverages. Biotechnology Advances, 1, 219-243.

Sui D.-Y, Liu T.-Q., Jiang X.-M., Ge D., Li X.-Q., Mu C.-F., Sun X.-Y.(2009). Ethanol fermentation process in series with gel immobilized cells and integrated with Membra Gao Xiao Hua Xue Gong Cheng Xue Bao. J. Chem. Eng. Chin. Univ. 23, 80-86.

Tamang JP.(2010). Diversity of fermented beverages and alcoholic drinks. In:Tamang JP and Kailasapathy K, editors. Fermented foods and beverages of the world. New York (NY): CRC Press;. p. 85e 125

Teshome D.A., Rainer M., Noel C.J., Schüßler G., Fuchs D., Bliem H.R., Günther B.K.(2017). Chemical compositions of traditional alcoholic beverages and consumers' characteristics, Ethiopia. African J.of Food Science, 11(7), 234-245

Urga K, Fite A, Biratu E. (1997). Effect of natural fermentation on nutritional and antinutritional factors of tef (Eragrostis tef). Ethiop. J. Health Dev. 11, 61-66.

Vaskova, (2013) Quantitative evaluation of methanol content in beverages based on raman spectral data. Mathematics Computers Biology Biomed informatics No volume: 81–85

Vogel, S. and Gobezie, A., (1983): Ethiopian ' tej'. In: Handbook of Indigenous Fermented Foods. Steinkraus KH (ed). Marcel Dekker, Inc. New York, pp 363-365.

WHO, (2004). Global status report on alcohol and health. Available http://www.who.int/substance_abuse/publications/global_status_report_2004_overview.pdf

WHO-AFRO, (2010). Reduction of the Harmful use of Alcohol, a Strategy for the WHO African Region.

WHO, (2004) World Health Organization. Global Status Report on Alcohol. Geneva (Switzerland).

WHO, (2011). World Health Organization. Department of Mental Health and Substance abuse. Global Status Report on Alcohol and Health, 2011. Geneva (Switzerland).

WHO, (2014). Global status report on alcohol and health.

WHO, (2014) World Health Organization. Global Status Report on Alcohol, Geneva (Switzerland).

Yohannes, T., Melak, F., and Siraj, K. (2013) Preparation and physicochemical analysis of some Ethiopian traditional alcoholic beverages. Afr. J. Food Sci7 (11) ,399- 403.

Zvauya, R., Mygochi, T., and Parawira, W. (1997): Microbiological and biochemical changes occurring during production of masvusvu and mangisi, traditional Zimbabwean beverages

7. APPENDEX- 1

Multiple Comparisons LSD

Dependent Variable		(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.
					Lower BounD	Upper Bound
PH	A	B	.020000	.065405	.768	-.13082 .17082
		c	.156667*	.065405	.043	.00584 .30749
		d	-.863333*	.065405	.000	-1.01416 -.71251
	B	A	-.020000	.065405	.768	-.17082 .13082
		c	.136667	.065405	.070	-.01416 .28749
		d	-.883333*	.065405	.000	-1.03416 -.73251
	c	A	-.156667*	.065405	.043	-.30749 -.00584
		B	-.136667	.065405	.070	-.28749 .01416
		d	-1.020000*	.065405	.000	-1.17082 -.86918
	d	A	.863333*	.065405	.000	.71251 1.01416
		B	.883333*	.065405	.000	.73251 1.03416
		c	1.020000*	.065405	.000	.86918 1.17082

* The mean difference is significant at the 0.05 level.

APPENDIX-2

Alcohols A	B	-.410000*	.030185	.000	-.4796	-.34039
	c	.713333*	.030185	.000	.64373	.78294
	d	3.503333*	.030185	.000	3.43373 3	.57294
B	A	.410000*	.030185	.000	.34039	.47961
	c	1.123333*	.030185	.000	1.05373	1.19294
	d	3.913333*	.030185	.000	3.84373	3.98294
c	A	-.713333*	.030185	.000	-.78294	-.64373
	B	-1.123333*	.030185	.000	-1.19294	-1.05373
	d	2.790000*	.030185	.000	2.72039	2.85961
d	A	-3.503333*	.030185	.000	-3.5729	-3.43373
	B	-3.913333*	.030185	.000	-3.98294	-3.84373
	c	-2.790000*	.030185	.000	-2.85961	-2.72039

* The mean difference is significant at the 0.05 level.

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14BTC IN EA CHLOROFORM14-15-40.D
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Inj Volume : 1 µl
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Analysis Method : C:\CHEM32_T\1\METHODS\ALCOHOLS.M
Last changed : 9/14/2018 4:00:20 PM by SYSTEM
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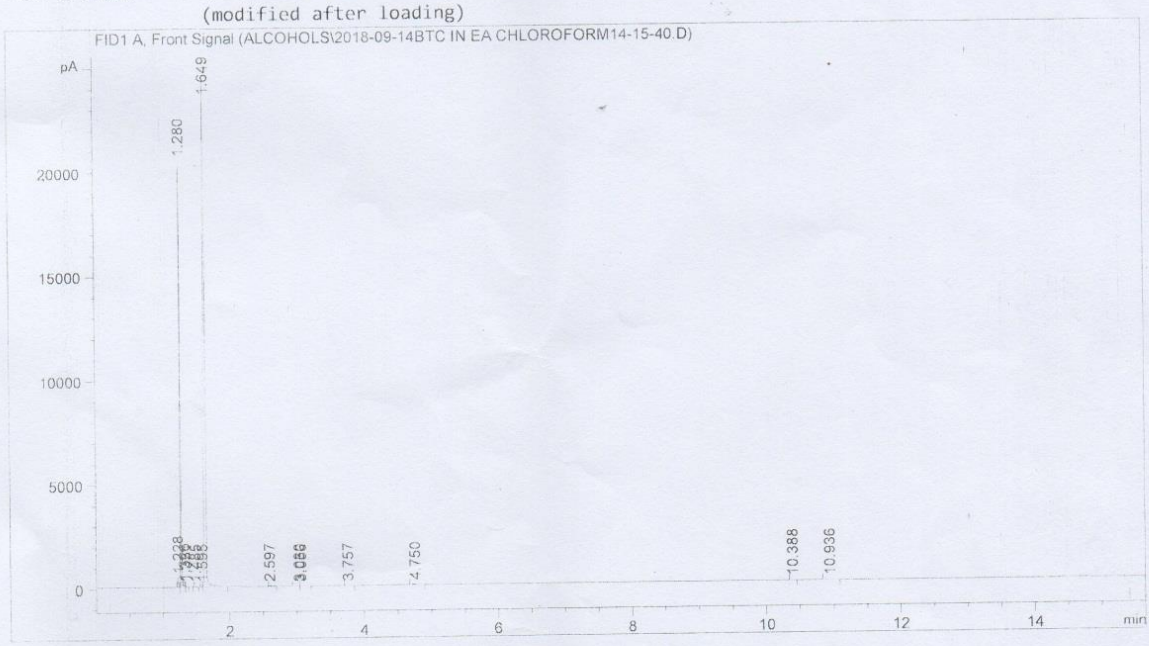

Area Percent Report

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Dilution : 1.0000
Sample Amount: : 9.45400e-1 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	1.228	BV	8.94e-3	241.37854	0.44760	Methanol
2	1.280	VB S	0.0106	1.29568e4	24.02628	Ethanol
3	1.326	BV X	0.0127	18.43825	0.03419	
4	1.351	VB X	8.28e-3	12.12874	0.02249	?
5	1.485	BB	0.0173	10.48839	0.01945	?

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14BTC IN EA CHLOROFORM14-15-40.D
Sample Name: BTC in EA chloroform

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
6	1.595	BV	0.0137	4.09792	0.00760	?
7	1.649	VB S	0.0285	4.06017e4	75.28931	chloroform
8	1.704		0.0000	0.00000	0.00000	
9	2.142		0.0000	0.00000	0.00000	Buthan-1-ol
10	2.597	BB	0.0391	41.06404	0.07615	?
11	2.768		0.0000	0.00000	0.00000	
12	3.030	BV	0.0529	6.41500	0.01190	?
13	3.056	VB	0.0798	9.09188	0.01686	?
14	3.757	BB	0.0504	4.45856	0.00827	?
15	4.750	BB	0.0527	10.23015	0.01897	?
16	10.388	BB	0.0286	2.41154	0.00447	?
17	10.936	BB	0.0773	8.88253	0.01647	?

Totals : 5.39276e4

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14MTC IN EA CHLOROFORM15-13-16.D
Sample Name: MTC in EA chloroform

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
6	1.651	BB S	0.0287	4.15282e4	78.00532	chloroform
7	1.704		0.0000	0.00000	0.00000	
8	2.142		0.0000	0.00000	0.00000	Buthan-1-ol
9	2.598	BB	0.0396	47.62909	0.08947	?
10	2.768		0.0000	0.00000	0.00000	

Totals : 5.32376e4

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Sample Name: MTC in EA chloroform

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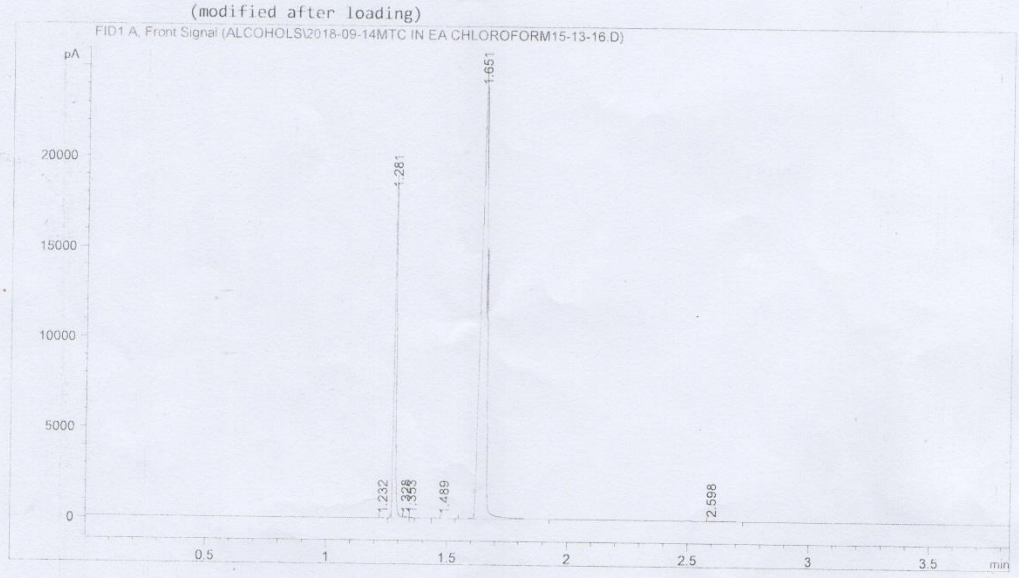

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Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	1.232	BB	8.93e-3	10.47354	0.01967	Methanol
2	1.281	BB S	0.0107	1.16014e4	21.79165	Ethanol
3	1.328	BV X	0.0138	25.20178	0.04734	
4	1.353	VB X	9.83e-3	15.77917	0.02964	?
5	1.489	BB	0.0162	9.00045	0.01691	?

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14B+MTC IN EA CHLOROFORM14-52-42.D
Sample Name: B+MTC in EA chloroform

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Area Percent Report

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Dilution : 1.0000
Sample Amount: : 6.99800e-1 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	0.813	BV	0.0130	3.16682	0.00627	?
2	1.232	BB	9.00e-3	3.58056	0.00709	Methanol
3	1.279	BB S	9.17e-3	7720.83643	15.28747	Ethanol
4	1.328	BV X	0.0144	26.64661	0.05276	
5	1.352	VB X	9.66e-3	17.73257	0.03511	?

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14B+MTC IN EA CHLOROFORM14-52-42.D
Sample Name: B+MTC in EA chloroform

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
6	1.489	BB	0.0182	8.26722	0.01637	?
7	1.651	BB S	0.0289	4.26951e4	84.53743	chloroform
8	1.704		0.0000	0.00000	0.00000	
9	2.142		0.0000	0.00000	0.00000	Buthan-1-ol
10	2.607	BB	0.0304	25.37624	0.05025	?
11	2.768		0.0000	0.00000	0.00000	
12	6.018	BV	0.0445	3.66456	0.00726	?
Totals :				5.05044e4		

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14PTC IN EA CHLOROFORM14-35-14.D
Sample Name: PTC in EA chloroform

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
6	1.487	BB	0.0184	9.33036	0.01789	?
7	1.596	BV	0.0135	4.51283	0.00865	?
8	1.651	VB S	0.0296	4.30462e4	82.53636	chloroform
9	1.704		0.0000	0.00000	0.00000	
10	2.142		0.0000	0.00000	0.00000	Buthan-1-ol
11	2.620	BB	0.0345	19.08161	0.03659	?
12	2.768		0.0000	0.00000	0.00000	
Totals :				5.21542e4		

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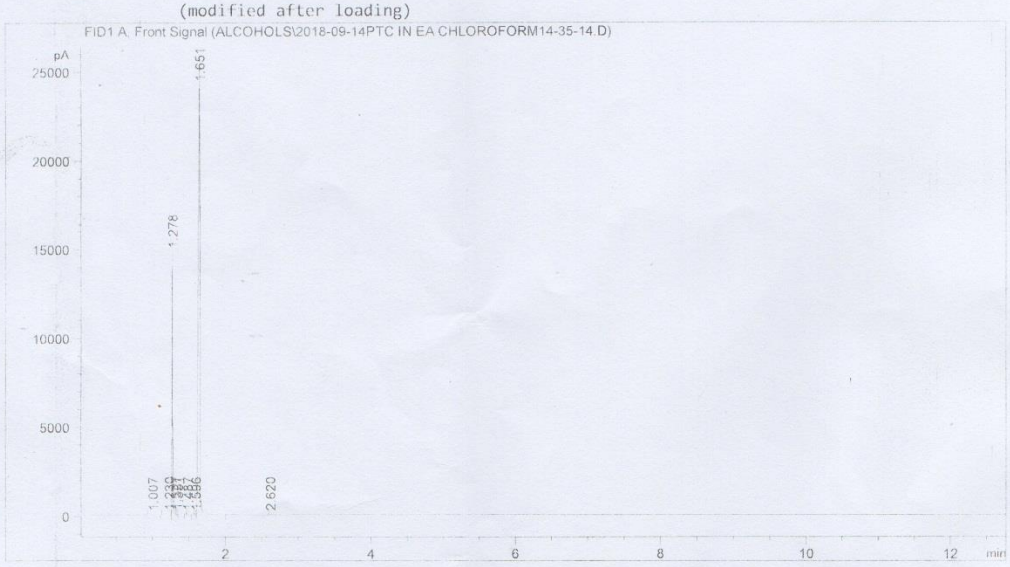

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Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	1.007	BB	0.0384	3.69723	0.00709	?
2	1.230	BV	0.0135	21.42016	0.04107	Methanol
3	1.278	VB S	0.0102	9010.45117	17.27656	Ethanol
4	1.327	BV X	0.0142	23.00798	0.04412	
5	1.351	VB X	0.0102	16.51805	0.03167	?

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14PAC IN EA CHLOROFORM15-02-53.D
Sample Name: PAC in EA chloroform

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Analysis Method : C:\CHEM32_T\1\METHODS\ALCOHOLS.M
Last changed : 9/14/2018 4:00:20 PM by SYSTEM
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Area Percent Report

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Dilution : 1.0000
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Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	1.167	BV	0.0606	12.08565	0.02322	?
2	1.231	VB	8.86e-3	5.37382	0.01032	Methanol
3	1.279	BB S	9.77e-3	9636.86230	18.51167	Ethanol
4	1.327	BV X	0.0138	25.38063	0.04875	
5	1.352	VB X	9.36e-3	16.17032	0.03106	?

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14PAC IN EA CHLOROFORM15-02-53.D
Sample Name: PAC in EA chloroform

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
6	1.488	BB	0.0173	8.79313	0.01689	?
7	1.596	BV	0.0146	2.52400	0.00485	?
8	1.651	VB S	0.0288	4.23025e4	81.25991	chloroform
9	1.704		0.0000	0.00000	0.00000	
10	2.142		0.0000	0.00000	0.00000	Buthan-1-ol
11	2.610	BB	0.0361	24.86465	0.04776	?
12	2.768		0.0000	0.00000	0.00000	
13	2.847	BB	0.0197	2.72511	0.00523	?
14	3.588	BB	0.0530	11.00365	0.02114	?
15	4.433	BB	0.0451	9.98861	0.01919	?
Totals :				5.20583e4		

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14METHANOL EA CHLOREF15-33-10.D
Sample Name: Methanol EA chloref

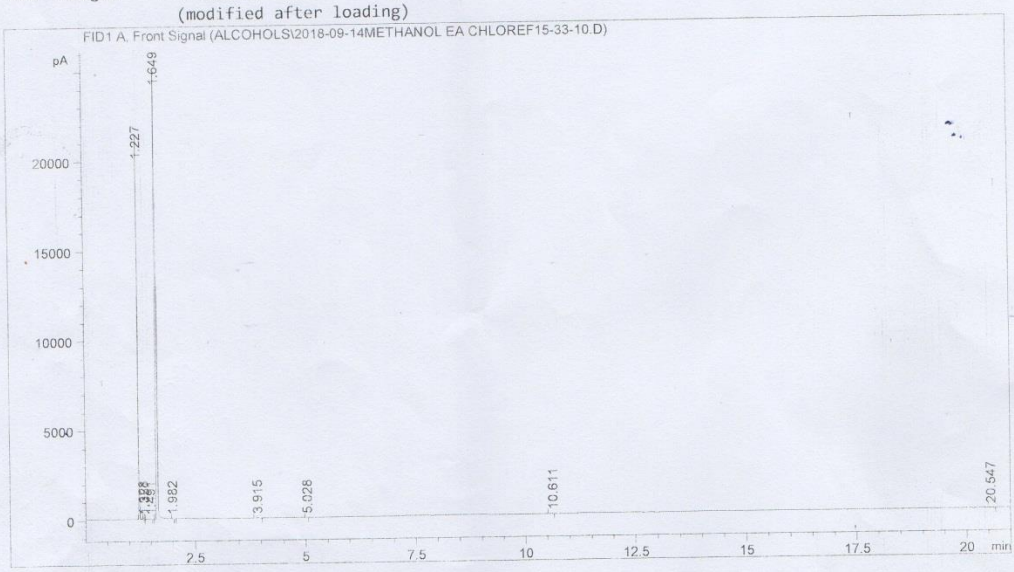
Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
6	1.649	BB S	0.0287	4.15495e4	79.08609	chloroform
7	1.704		0.0000	0.00000	0.00000	
8	1.982	BB X	0.0195	3.51054	0.00668	?
9	2.142		0.0000	0.00000	0.00000	Buthan-1-ol
10	2.768		0.0000	0.00000	0.00000	
11	3.915	BB	0.0521	11.81547	0.02249	?
12	5.028	BV	0.0329	2.59581	0.00494	?
13	10.611	BV	0.0626	6.93452	0.01320	?
14	20.547	BB	0.0518	4.77294	0.00908	?
Totals :				5.25371e4		

Data File C:\CHEM32_T\1\DATA\ALCOHOLS\2018-09-14METHANOL EA CHLOREF15-33-10.D
Sample Name: Methanol EA chloref

Reference grade Methanol
Purity 99.9 %
Wt = 0.955 gm

Acq. Operator : SYSTEM
Acq. Instrument : Agilent 7890 GC Location : Vial 6
Injection Date : 9/14/2018 3:34:44 PM
Inj Volume : 1 µl

Acq. Method : C:\CHEM32_T\1\METHODS\ALCOHOLS.M
Last changed : 9/14/2018 11:02:05 AM by SYSTEM
(modified after loading)
Analysis Method : C:\CHEM32_T\1\METHODS\ALCOHOLS.M
Last changed : 9/14/2018 4:00:20 PM by SYSTEM
(modified after loading)



Area Percent Report

Sorted By : Signal
Calib. Data Modified : 9/14/2018 4:00:05 PM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount: : 9.51000e-1 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
1	1.227	BB S	8.00e-3	1.08850e4	20.71869	Methanol
2	1.297		0.0000	0.00000	0.00000	Ethanol
3	1.328	BV X	0.0141	39.76907	0.07570	
4	1.351	VV X	0.0125	23.62072	0.04496	?
5	1.491	VB X	0.0587	9.54468	0.01817	?