ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

DETERMINATION OF ETHANOL LEVEL IN BEVERAGES

BY
Gizaw Debebe

July, 2006

DETERMINATION OF ETHANOL LEVEL IN BEVERAGES

A Graduate project Submitted to the
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In Partial Fulfillment of the Requirement for the
Degree of Master of Science in Chemistry

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DECLARATION

This project is my original work except where due reference has been made in the acknowledgments. This work has not been submitted for a degree in any other University.

Signature --------------------

Date ----------------

This project has been submitted for examination with my approval as university advisor.

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Abstract

Some of the samples of Ethiopian traditional alcoholic beverages namely 'tella', 'tej' and 'areki' collected from different parts of Addis Ababa were analyzed for their alcohol contents. The alcohol contents of 'tella' and 'tej' were analyzed by adopting the method of determination of ethanol level in beer. The alcohol content of 'areki' was determined by using an alcoholometer. The average ethanol content (%v/v) of the traditional alcoholic beverage was found to be 6.36, 11.47, and 37.22 for 'tella', 'tej', and 'areki', respectively.

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I. Introduction

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 tens thousands of years ago. 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 [Alcoholic Beverages, The Free Encyclopedia]. According to the report of F. O. Olandiole, [2002], however, alcoholic beverages have been used since the landing of Pilgrims.

The oldest alcoholic drinks were fermented beverages of relatively low alcoholic contents [Rose, 1977]. Before the advent of the distillation technique as introduced into Europe by Arabs, the oldest alcoholic drinks were fermented beverages of relatively low alcohol content, such as beers and wines [Desta B., 1977]. The development of distilled alcoholic beverage was associated with the advent and improvement of distillation techniques.

Fermentation is one of the effective and most economical method of processing foods and beverages acceptable to man. The method is inexpensive, easily acceptable, and adaptable at household level in traditional communities [Pederson, 1979; Nout 1993]. Fermentation processes enhance the nutritional quality of raw ingredient by improving the digestibility of nutrients and inactivating anti-nutritional factors [Van Veen and Steinkraus, 1970; Nout 1993]. They also improve acceptability of the food by destroying undesirable flavors of the raw ingredients [Steinkraus, 1983].

Fermentation is a widely practiced ancient technology and fermented foods are an essential part of diets in all regions of the world. Traditional fermented beverages are those that are indigenous to a particular area and have been developed by the local people using an age-old techniques and locally available raw materials [Kebede *et al*., 2002]. Practically, every civilization has developed some type of fermented food and beverage. The early men probably used fermented beverage as a substitute for save 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 [Rose, 1977].

Ethanol is the only type of alcohol that can be consumed. Commercial ethanol for consumption is prepared from various forms of starch and sugars by fermentations. Ethanol affects the central nervous system, gastro-intestinal tract, cardiovascular system, endocrine, liver, lipid metabolism, fetal development, and has immune suppression activities. Alcohol is absorbed into a blood via stomach and/or intestine depending on the amount of alcohol consumed. About 90-98% is metabolized in the liver into acetic acid and 2-10% is excreted unchanged. There are several damaging effects of alcoholism such as mental problems, job trouble, frequent blockouts, loss of control etc. [Olandiole, 2002].

II. Literature review

Fermented alcoholic beverages around the world are consumed in different occasions. In nearly all areas of the world, some type of alcoholic beverages native to its region is prepared and consumed [Banwert, 1983]. Fermented beverages vary considerably in type. Fermented beverages produced from cereals usually referred to as beers while those produced from fruits are classified as wines [Pederson, 1979].

Indigenous fermented alcoholic beverages from different parts of the world are described [Steinkraus 1983]. Among these, information on the microbiology and biochemical properties of varieties of the indigenous African fermented alcoholic beverages is available. These include Egyptian *bouza*, Tanzanian *wanzuki*, *gongo, tembo-mnazi* and *gara*, Nigerian *palm-wine*, Kenyan *muratna* and *uragela*, and South African *kaffir* beer [Ashenafi *et al*., 2001]. Indigenous Ethiopian fermented beverages include *tej* [Vogel and Gobezie 1983; Fite, *et al*., 1991; Bahiru B., 2000] *tella* [Sahle and Gashe, 1991], *borde* and *shamita* [Mogessie A. and Tetemke M., 1995; Bacha *et al*., 1998, 1999], *araki* [Desta B., 1977; Fite, *et al*., 1991].

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 [Harkishore, 1977]. Palm wines (*Toddys*) are fermented and consumed under different parts of the world. Palm wine has 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 pants [Okafer, 1972].

There has been an increase in cancer risk in people consuming alcoholic beverages, and hence there has been a suspect that consumption of alcoholic beverages is to be a carcinogen. However, studies specifically examining the carcinogenicity of ethanol in the experimental animals have not yielded results that would suggest that the ethanol component of alcoholic beverages is solely responsible for the increase in cancer observed in people consuming alcoholic beverages. Evidence supports a weaker, but possibly causal, relation between alcoholic beverages consumption and increased risk of cancers of the liver and breast [Lognecker, 1994].

It has often been observed that alcoholism is a more significant problem than all other forms of drug abuse combined [Desta B., 1977]. According to some studies carried out in some African countries there is considerable evidence that home-produced alcohol drinks are known to have toxic components [Fite *et al*., 1991]. A report from Zambia, as quoted by Fite *et al*., [1991], indicates that moulds such as *Mucor* could frequently be found on the fermenting source of pectinase, the enzyme that breaks down pectinto release methanol. Methanol and fusel oil were shown to be the common contaminants of traditional alcoholic beverages in the studies carried out so far.

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

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

Of traditionally fermented beverages in Ethiopia, the most popular alcoholic beverages are *tej* (honey wine), *tella* (a malt beverage like beer) and *areki* (distilled liquor). These drinks are widely served on festive occasions and at social gatherings. According to a census conducted in 1988, about 11,000 persons were then engaged in the trade of traditional beverages in Addis Ababa alone [Central Statistics Authority, 1988]. This is about 32% of the population of the city engaged in internal trade.

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

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

1. Ethiopian traditional alcoholic beverages

Tella

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

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

Tella is made from different cereals. *Tef* and corn are the most popular, but in some areas barely, millet or sorghum can be used [Selinus R., 1971]. The way of preparing *tella* differs among the ethnic groups and depends on traditional and the economic situation. The clay container (*insera*) is washed with *Grawa (Vernonia amygdalina)* and water several times and after that smoked with wood from *Weyra (Olea europaea subsp. Cuspidate)* for about ten minutes, in order to get it as clean as possible. Germinated grain of barley, or corn, or wheat *(bikil*), bought in the local market or prepared at home, are dried and milled. For making *bikil*, the grains are moistened in water and the moist grains are placed between fresh leaves, left to germinate for 3 days and after that dried. *Gesho*

(*Rhamnus prenoids*), local hops, is available dried in the local market. The leaves *gesho* (*Rhamnus prenoids*) is dried again in the sun for about ½ hour and after that pounded. The leaves are separated from the stems, which need a longer time to dry. The ground *gesho* (*Rhamnus prenoids*) leaves are placed in a clay container with water and left to ferment for 2-3 days. *Gesho* (*Rhamnus prenoids*) is responsible for the bitter taste of *tella* and the bitterness of *tella* is directly related to the amount of *gesho* added during brewing. It is also thought to be the source of various chemicals [Shale and Gashe, 1991]. It is assumed that *gesho* (*Rhamnus prenoids*) maintains acidic pH during *tella* fermentation so as to modify the nature of the mash and inhibits the growth of undesirable microorganism [Kebede T., 1994]. Some of the grains intended for *tella* preparation are toasted and milled, and then mixed with water and baked on the *mitad* to prepare what is known as *kita* (a thin, 5-10 mm thick, pancake-like bread),. This *kita*, broken into small pieces, part of the milled *bikil* and the pounded *gesho* stems are added to the water and allowed to ferment for 1-2 days. The rest of the flour is toasted on *mitad,* sprinkled with water and toasted until dark brown to form what is known as *enkuro*. This mixture *enkuro*, the rest of the germinated grains (*bikil*), some *gesho* (*Rhamnus prenoids*), and water are added to the container. The mixture is kept covered overnight, after which more water is added and the container is kept sealed for 5-7 days, until when the beverage is ready. *Tella* can be kept for 10-12 days. High quality *tella* is made with a relatively small quantity of water.

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

For *tella* considered to be a good quality, the final ethanol content is in the range of 2-8% (v/v) and pH is 4-5 [Sahle and Gashe, 1991]. The biochemical changes, the microorganisms involved in the fermentation and those which bring about desirable and undesirable changes in the process of *tella* making are described [Sahle and Gashe, 1991]. According to the report, the fermentation process of *tella* is divided into four phases. The first occurs in the original mixtures of ingredients, and the second and third phases occur after successive additions of more carbohydrate materials. The three main carbohydrate materials are mentioned to be *bikil, kitta* and *enkuro*. The latter phase is where acidification takes place, which is actually not desirable. Maximum ethanol production occurs during the third phase and at the beginning of the fourth phase.

The report of Sahle and Gashe, [1991], also indicates that the extent of heat treatment the *asharo* (roasted barley) receives and the degree of steaming the *enkuro* (roasted barley steamed after grinding) is subjected to have the direct bearing on the color of *tella*, which is determined by the housewife preparing the *tella. Tella* is actually a beverage of variable viscosity and having a variety of colors (ranging from grayish-white to dark brown).

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

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

Kirari is a drink made, when the clear *tella* is used, by adding fresh water and then leaving the mixture to ferment. This beverage is weaker than the regular *tella*, and is most often used for family consumption.

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]. Some also add different concoctions such as barks or roots of some plants or secrete herbal ingredients to improve flavor or potency and to attract customers. Due to concoction, adulteration practices and possibly some other reasons, producers usually are not willing to tell about additives used and their composition [Bahiru B., 2000].

According to Vogel and Gobezie, [1983], during the preparation of *tej*, the fermentation pot is seasoned by smoking over smoldering *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 *Rhamnus prenoides* and put back to the fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15-20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *Rhamnus prenoides*.

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

Fermentation of *tej*, like other traditionally fermented alcoholic beverages, relies on the microorganisms present in the substrates, fermentation vats or equipment. As these fermentations are natural and, thus, uncontrolled, alcohol and fusel oils produced during the fermentation can be hazardous to health if produced beyond acceptable levels. With the variable microflora of such spontaneous fermentation, variability of the product is inevitable [Bahiru B., 2000].

The ethanol content of *tej* is reported to range from 13.2% to 13.7% [Desta B., 1977], 7% to 14% [Vogel & Gobezie, 1983], 6.2% (V/V) with significant variations among samples [Fite *et al*., 1991], and 9.07%, again with significant variations [Bahiru B., 2000]. The mean value of methanol and fusel oil, for *tej* samples collected from different regions of Ethiopia were 47 ppm and 104 ppm, respectively. According Ashenafi *et al*., [2001], the fusel oil content of '*tej*' samples varies between 0.1 g/l00L and 88 g/100L.

Shamita

Shamita is another traditional beverage 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]. *Shamita* is a widely consumed beverage in different regions of Ethiopia. It has a thick consistency and most people who cannot afford a reasonable meal consume it as meal replacement. It is produced by fermenting roasted barely overnight. Malt is not commonly used in *shamita* fermentation, although local *shamita* brewers in Addis Ababa use it frequently, and starch is the only principal fermentable carbohydrate.

The microorganisms responsible for fermentation are mostly from back slopping using small amount of *shamita* from a previous fermentation as well as from the ingredients and equipment. Ready to consume *shamita* 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, *shamita* 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 A. and Tetemke M., 1995].

Borde

Borde is a traditional fermented beverage of Ethiopia, a common meal replacement in Southern Ethiopia, and some other parts of the country [Mogessie A. and Tetemke M., 1995]. According to the report of Mogessie A. and Tetemke M., [1995], it is consumed while actively fermenting and has a short fermentation period, usually overnight. On the average, a laborer consumes about three liters of *borde* per day.

Borde is prepared from unmalted maize (*Zea mays*), barley (*Hordeum vuldare*), wheat (*Triticum sativum*), finger millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*) and/or *tef* (*Eragrostis tef*) and their malt, except sorghum and *tef* [Kebede *et al*., 2002]. *Tef* is less frequently used for *borde* preparation may be due to its high cost and preference for use in *injera* (pancake-like bread). The type of cereal and amount of malt utilized for *borde* preparation varies both within and between localities. Maize, followed by barley and wheat, was found to be the most common ingredient of *borde* both as malt and unmalted ingredient in southern Ethiopia, whereas wheat has been reported to be the preferred unmalted ingredient in Addis Ababa [Bacha K., 1997]. As reported by Kebede *et al*., [2002], some brewers in Awassa and at Bedessa used both finger millet and sorghum as unmalted ingredient, whereas some at Gununo and Sodo Zuria used sorghum but not finger millet. Sorghum was used as unmalted ingredient but not as malt in the study areas. Finger millet was more frequently utilized for malt preparation than as unmalted ingredient. The ingredients used and their possible combinations were found to vary within and between localities and are selected according to availability, price and preference. Seasonal variations in the price of the various cereals also affect the choice of ingredients [Kebede *et al*., 2002].

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 (Capsicum minimum), 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].

Areki

Areki 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*., 1991]. *Areki* 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 *areki* are predominantly lower class people or those who have become dependent on alcohol and cannot afford to buy industrially produced alcohol [WHO, 2004].

Traditionally *areki* is classified into two: *Terra-areki* and *Dagim-areki*. The term *dagim* in Amharic refers to ‘second time’ and, indicates that it is distilled second time, whereas the term *terra* in Amharic refers to ‘ordinary’.

Terra-areki

Terra-areki is a colorless, clear, local alcoholic beverage, which is distilled from a fermentation product known as *Yereki-tinsis* [Desta B., 1977]. According to the report of Desta B., [1977], *yereki-tensis* is prepared by mixing powdered *Gesho* leaves and powdered *bikil* (1:2 ratios) with water to give a mixiture of free-flowing consistency, and which will be put aside to ferment for about five days. An amount of *Dagussa* (Elusine coracann) roughly equivalent to four times that of the *bikil*, is powdered kneaded with water to make a 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-areki*. The alcohol content of *terra-areki* was reported to be 34.09% (v/v) [Desta B., 1977], and varies between 22.0 – 28.0% (v/v) [Selinus R., 1971].

Dagim-areki

Dagim-areki is a stronger type of *terra-areki*, which is prepared in the same way as *terra-areki*, 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-areki* [Desta B., 1977]. The redistilled *areki* will then have higher alcohol content. The average alcohol content of *dagim areki* is around 45% (v/v) [Selinus R, 1971]. It was also reported to have a mean value of 46.6% (v/v) ethanol content [Desta B., 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 [Selinus R, 1971]. 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].

According to the report of Sanni, [1992], as mentioned by Bahiru B., [2000], fermented foods and beverages constitute a major protein of people's diet in Africa. However, preparations of those products are still traditional family arts and the fermentation is by uncontrolled inoculation. Mixed-culture of spontaneous fermentation is the rule in African food fermentation and the disadvantages of mixed-culture and uncontrolled fermentation is the recurring variations in product quality. 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].

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]. Therefore, 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.

III. Objective

The main objective of this project is to determine the alcoholic contents of beverages using different chemical and instrumental methods.

IV. Materials and methods

1. Sampling

Samples of *tella, tej*, and *areki* from different places of Addis Ababa were considered in this study. A sample of *dagim-areki*, which was originally from Gojam, was also included in the study.

A total of five samples of *tella* were obtained from different sources. One sample was provided by Prof. Ermias Dagne, which was prepared in his own home. The other one sample was provided by Dr. Gizachew Alemayehu. The other two samples of *tella* were provided by two of my colleagues; Kibrom G/Hiyiwot and Getahun Asmare. The remaining one sample was collected from the house of one resident in *Lideta* sub-city, Addis Ababa.

A total of five samples of *tej* were collected from five different vending houses in Addis Ababa, i.e. one sample from each vending house. The vending houses are selected randomly. The prices of the samples of *tej* vary considerably; ranging from birr 3 to birr 13 per liter. The samples purchased from Addis Ababa restaurant and '*Tobiya tej bet*' are costly.

Samples of *terra-areki* were collected from retail sellers in Addis Ababa. Again the selection is random. A total of five samples of *terra-areki* were considered in this study. Prof. Ermias Dagne provided one sample of *dagim-areki*, which was originally from Gojam. All the samples were collected in screw-capped plastic bottles (bottles of highland springs). The samples were kept in a refrigerator right after collection and until the biochemical analysis.

2. Chemical analysis

All the chemical analyses were done in the laboratories of National Alcohol and Liquor Factory and the quality control of St. George Brewery, except GC analysis, which was conducted in Addis Ababa University, Department of Chemistry.

pH values of the samples

The pH of the samples was measured by dipping the electrode of a digital pH meter (SCHOTT CG 837) to the samples.

Determination of ethanol level of the samples

The ethanol level of *tella* and *tej* was determined in the quality control laboratory of St. George Brewery, Addis Ababa. The assumption made here was that the method of determination of ethanol level in beer can be applied to the local drinks: *tella* and *tej*. Ethanol level of *areki* was determined in the National Alcohol and Liquor Factory, Addis Ababa.

Determination of ethanol level of *tella* and *tej*

The determination of ethanol level of tella and tej involves measurement of refractive index and specific gravity. Prior to any measurements, the samples were heated in a water bath (50 °C) and then filtered using fluted filter paper. The temperature of the filtrate was then set at 20 °C. The refractive index and the specific gravity of the samples were measured at this temperature (20 °C).

Determination of the refractive index

All the refractive indices were measured by using a refractometer (Gena 1 with prism L1) on to which a water bath and thermostat (Julabo EC) are attached. The function of the water bath and the thermostat is to keep the temperature of the system constant (at 20 °C). Before and after each measurement of the refractive index of each sample, the refractometer was calibrated with distilled water.

Determination of the specific gravity

The specific gravity of the samples was determined using a pycnometer (long neck pycnometer 223 and 245). The weights of an empty pycnometer, pycnometer with distilled water, and pycnometer with the samples were measured using a balance (Sartorius BP211D).

The specific gravity was then calculated from the weight of the empty pycnometer, pycnometer with distilled water, and pycnometer with the samples, as follow:

$$\text{Specific Gravity} = \frac{\text{Weight of pycnometr with a sample} - \text{Weight of empty pycnometer}}{\text{Weight of distilled water}}$$

The alcoholic content of the samples were calculated from regression equation derived in BGI Ethiopia, St. George Brewery.

The regression equation is:

Alcoholic content by weight [A (%w/w)]= [0.2965*RI – 295.8*SG + 291.285 – 0.04]

Where RI is refractive index and SG is specific gravity.

Alcoholic content by volume:

$$[A(\%v/v)] = \frac{[A(\%\ w/w)] \times SG}{0.791}$$

Determination of ethanol level of distilled liquor (*areki*)

The ethanol level of distilled liquor (*areki*) was measured by using an alcoholometer (Alcoolometer Guy Lussac + 15 °C), in the laboratory of National Alcohol and Liquor Factory.

An alcoholometer is a special type of hydrometer, which is used for determining the alcoholic strength of liquids. Such types of hydrometers have special scale marked by volume percents of an alcohol in water.

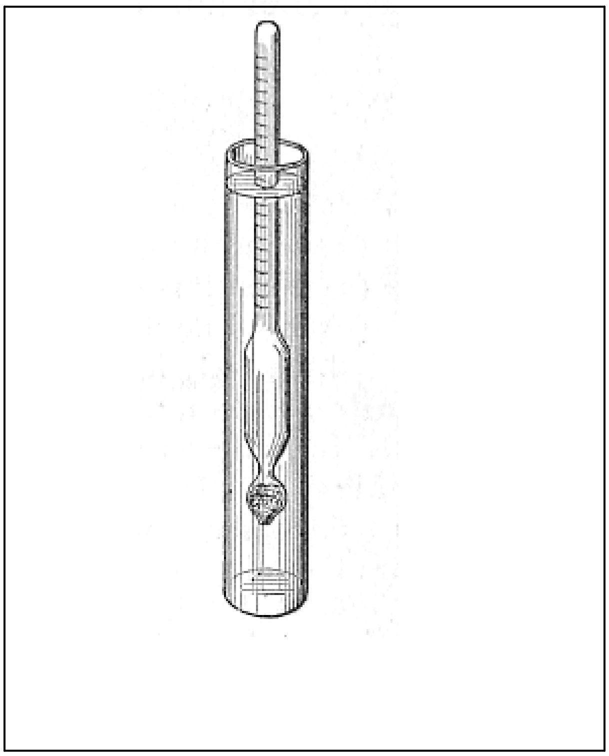

Fig. 1, schematic representation of a hydrometer

A hydrometer (Figure 1) is an instrument used for determining the specific gravity of liquids. It is usually made of glass and consists of a cylindrical stem and a bulb weighted with mercury or shot to make it float upright. The liquid is poured into a tall jar, and the hydrometer is gently lowered into the liquid until it floats freely.

The experiment was standardized by using the apparatus in the alcohol determination of samples of known alcohol content. The experiment for distilled liquor (*areki*) was conducted using 96% (v/v) ethanol and serial dilution of the 96% (v/v) ethanol. Taking a sample of St. George beer, diluted samples of St. George beer, and concentrated samples of St. George beer; the experiments for *tella* and *tej* were standardized.

Determination of methanol level and other volatile components of the samples

The determination of methanol level and other volatile compounds in the beverages was attempted by Gas chromatography (HP 6890). Prior to GC analysis the samples were distilled using a Clevenger apparatus (Figure 2).

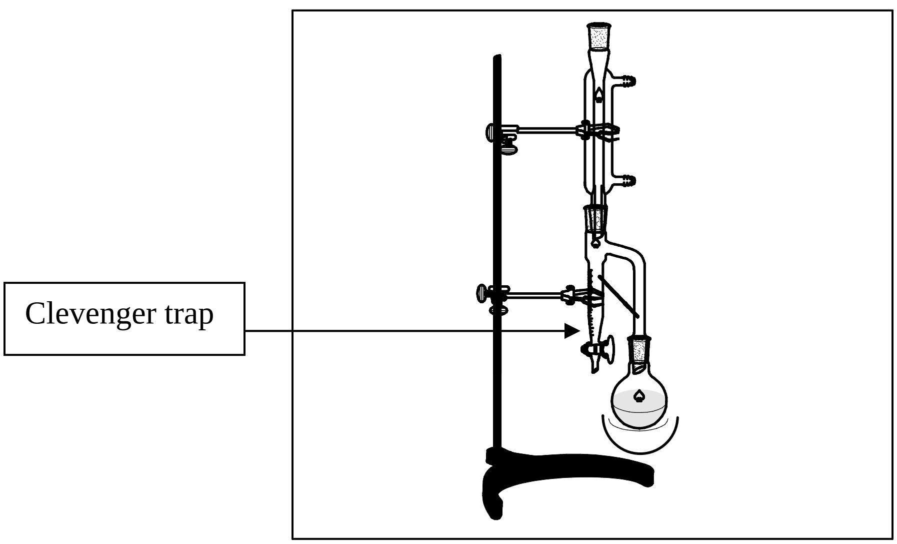

Figure 2: Schematic representation of a Clevenger apparatus (heavier than water)

To a round bottom flask, 15ml of the sample and standard solution [0.63% (v/v) acetone] were added and then distilled. This was done for each beverage sample. One milliliter of the distilled sample from the Clevenger trap was collected. To the collected distilled samples, one spatula of Na_2SO_4 was added as dehydrating agent.

The samples were then analyzed by GC (two experiment were done: one was done by diluting the samples and the other was done neat) with condition as follow: GC Model: HP 6890, Method: HP 5, GC Condition: Column type HP-5; Oven temperature Programmed from 50 – 70^{o}C, Detector Temp: 210^{o}C, Carrier gas Pressure: 2.5 ml/min, flow 0.5ml/min.

The minimum detectable levels (MDL) for some common detectors have been reported [Robert L. Grob, 1995]. The following table (Table 1) indicates the MDLs for some common detectors cited by Robert L. Grob, [1995].

Table 1: Minimum detectable level for some common detectors

Detectors	MDL	Sample compounds
Thermal conductivity	5×10^{-10} g/ml	Propane
Flame Ionization	10^{-12} g/s	Propane
Electron Capture	10^{-16} mol/ml	Lindane
Flame photometric	10^{-10} g/s	Thiophine
	2×10^{-10} g/s	Tributylphosphate

V. Result

1. Chemical properties of the beverages

1.1. pH values

Table 2, below, shows the pH of the samples. The pH value of *tella* ranged from 3.87 to 5.03, and that of *tej* varies between 3.74 and 3.79. Significant variations in pH values of the tella samples were noted. It can also be seen that *tej* is more acidic than *tella.*

Table 2: The pH of the Samples

	pH Values		
Samples	Tej	Tella	Kirari*
1	3.79	4.78	4.14
2	3.79	4.63	
3	3.74	4.14	
4	3.79	3.87	
5	3.75	5.03	
Average	3.77	4.51	

* Only one sample of Kirari was considered.

Table 3 and 4 show the data for the true alcohol value and the experimental alcohol values. The results of the control experiments indicate that there is a close agreement between the measured values and true values. This shows that the method is fairly accurate enough to determine the alcoholic content of the beverages.

Table 3: Data of the control experiment for *areki*.

Samples	Measured Value (%v/v)	True Value (% v/v)
1.	95.5	96
2.	48.8	48
3.	25.8	24
4.	12.8	12
5.	5.0	6

Table 4: Data of the control experiment for *tella* and *tej*.

Samples	Specific Gravity	Refractive Index	Measured Value (%v/v)	True Value (% v/v)
1.	1.00201	20.12	1.06	1.13
2.	1.00276	21.92	1.46	1.50
3.	1.00406	25.43	2.29	2.25
4.	1.00209	40.55	8.66	9.00
5.	0.99889	46.32	12.01	13.50
6.	0.99068	54.26	18.03	18.00
7.	1.00836	36.24	4.74	4.50 (St. George Beer)

1.2. The ethanol contents

The alcohol content of the traditional beverages is shown in tables 5 and 6.

Table 5: Percent ethanol content of traditional beverages (*tella* and *tej*)

Beverages	No. of Samples	Specific Gravity	Refractive Index	Alcohol Contents (%v/v)	Averages
Tella	1	1.00816	44.75	8.03	6.36
	2	1.00933	40.95	6.15	
	3	1.00834	42.05	6.93	
	4	1.00093	30.18	5.21	
	5	1.00081	30.60	5.46	
Tej	1	1.02371	74.80	13.75	11.47
	2	1.00228	47.00	11.02	
	3	1.00455	43.66	8.91	
	4	0.99823	47.68	12.76	
	5	1.00315	47.49	10.90	
Kirari*		1.00346	32.10	4.99	

* Only one sample was considered.

Table 6: Percent ethanol content of traditional beverages (*areki*)

Beverages	No. of Samples	Alcohol Contents (%v/v)	Averages
Terra-areki	1	39.80	37.22
	2	38.30	
	3	30.50	
	4	39.50	
	5	38.00	
Dagim-areki*		48.00	

*Only one sample was considered.

The overall mean alcoholic content of the samples of *tej* is 11.47 % (v/v) with a range of 8.91 - 13.75. The average alcoholic content of the sample of *tella* is 6.36% (v/v) with a range of 5.21 - 8.03. The alcoholic content of *terra-areki* varies from 30.50 - 39.80% (v/v) with the average value of 37.22% (v/v).

1.3. The methanol and other volatile compounds contents

There were no methanol and any other volatile compounds detected in the samples of *areki* that was considered in this study. The samples of *tella* and *tej* were also analyzed for their methanol contents and for other volatile compounds, if any, by GC. Again no methanol and other volatile compounds (e.g. aldehydes) were detected in the samples. The GC chromatograms are indicated in the appendices.

VI. Discussion

In this study it was found that the mean alcoholic content of the samples of *tej* is 11.47% (v/v) with a range of 8.91 to 13.75. The average alcoholic content of the sample of *tella* is 6.36% (v/v) with a range of 5.21 - 8.03. The alcoholic content of *terra-areki* varies from 30.5 - 39.8% (v/v) with the average value of 37.22% (v/v). This ethanol value for *terra- areki* is higher than the reported value. The reported mean value for *terra-areki* is 34.09. The highest value of ethanol in *Dagim-areki* is reported to be 47.59% (v/v) with a mean value and range of 46.60 and 45.72 – 47.59% (v/v) [Desta B., 1977]. The ethanol level of *dagim-areki* that was considered in this study was found to be 48.00% (v/v).

The alcoholic content of some of the beverages (Table 4) that are most frequently consumed by the masses is very high. According to Desta B., [1977], a comparison of traditional beverage with those that are industrially produced (locally) indicates a potency of similar magnitude. There is a significant difference in alcoholic content between the various traditional beverages [Desta B., 1977]. Bahiru B., [2000], reported a significant variation in the alcoholic content of *tej* samples collected from different places of Addis Ababa. According to the report the alcoholic content of *tej* is 9.07% (v/v) with a range of 0.32 to 21.72% (v/v).

The mean alcoholic content of Ethiopian traditional beverage varies from report to report. Desta B., [1977], 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.

Fite *et al*., [1991], had reported 6.57% (v/v) as a mean ethanol value of *tej* samples from three different regions (Gojam, Debre Berhan and Addis Ababa) of Ethiopia. They also reported that the *tej* samples collected from Addis Ababa have an average ethanol content of 7.5% (v/v) with range of 6.6 to 8.4% (v/v). The report also indicated 3.81% (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. The report again indicated that the samples of *tella* collected from Addis Ababa have an average ethanol content of 2.4% (v/v) with range of 1.6 to 2.8% (v/v). The variation in ethanol contents of the beverages is due to differences in preparation and fermentation [Fite, *et al*., 1991]. Conditions such as temperature, aeration, and strains of the microorganisms obviously affect the level of the alcohols [Fite, *et al*., 1991].

According to Sanni, [1992], as cited in Bahiru B., [2000], honey wine of Eastern Europe had an alcoholic content of 6.4 to 16.6% (v/v). 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 Gobezie, [1983], reported that if fermentation of *tej* goes to completion, the final alcoholic content would be 7 to 13% (v/v).

In comparison to the above beverages, the mean alcohol content of *tej* in this study is above that of the report of Fite *et al*., [1991], and it is close to the range of that of meads. But, the mean alcohol content of *tej* in this study is below, the mean alcohol content of honey wines in the US market and *tej* as reported by Desta B., [1977]. The mean alcohol content of *tella* in this study is above that of the *tella* reported by Desta B., [1977] and below the *tella* that reported by Fite *et al*., [1991].

The alcohol content of *dagim-areki* is relatively high, 48.00% (v/v), and even *terra-areki* has high mean alcohol content, 37.22% (v/v), with a range of 30.20 - 39.80% (v/v). The relatively high alcohol content of the traditional distilled liquor (*areki*) is a matter of concern in terms of alcoholism that may be rapidly introduced and also considering the high and ever expanding production and consumption of these products [Desta B., 1977].

In this study, no methanol contents in the samples of *areki, tella, and areki* were detected. In the studies conducted so far on the local beverages, however, the methanol content was reported in the ppm level; *areki* (320.87 ppm), *tella* (32.37 ppm), *tej* (45.67 ppm) [Fite *et al*., 1991].

Comparison of the literature value of methanol content of the local beverages and the values of the minimum detectable level of the common detectors of GC (Table 1), there is no methanol in the considered samples of the beverages.

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 [Desta B., 1977].

Special problems associated with chronic alcoholism, which are now thought to be related to nutritional deficiencies that are in turn, the result of the capacity of alcohol to supply calories and depress appetite without supplying vitamins and amino acids, have been observed [Victor, 1958].

VII. Conclusion and recommendations

The study indicated that the alcoholic content of Ethiopian traditional beverages vary considerably. The alcoholic content variability of local beverages could be attributed to the spontaneous fermentation.

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. However, care should be taken if modernization is to be considered. According to Novellie, [1968], as cited by Bacha K., [1997], Kenyan *busaa* produced by modern techniques has lost acceptance by those accustomed to traditional *busaa*. Kaffir beer has shown dramatic decrease in vitamin values following modernization. This means that while developing successful modern techniques, traditional processes should not be overlooked and rather should be utilized by making them more hygienic.

Some traditional fermented beverages are already scaled-up. Urbanization and industrialization have been the driving force behind the large-scale development of some traditional fermented beverages [Bacha K., 1997]. In Nigeria, the Federal Institute of Industrial Research successfully introduced bottled and preserved palm wine [Nout, 1980]. In modernization, the cost of product should be given special attention. Refining, bottling or canning would obviously end-up with rise in cost of the products. This might limit the drinks to those with more purchasing power.

In Ethiopia the government has no control over the production and quality of traditional beverages. There is, however, wide spreading and serious alcohol-related problems. Thus, control in the production and supervision with the development of comprehensive national alcohol policy is recommended.

It is not difficult to imagine that large number of population in the most parts of the country consumes the local beverages. As the process of fermentation is spontaneous, high amount and variability of toxic substances is inevitable. Hence from the point of view of public health, investigation on the mechanism of production and means to avoid predominance of harmful contents are necessary.

Further researches need to be conducted toward the development of standardized ways of production. Estimation of the amount of the harmful components of the traditional beverages, in relation to human health, is required. It is also important to deal with these harmful components so as to assess whether they are found in the beverages above or below their maximum allowable concentrations.

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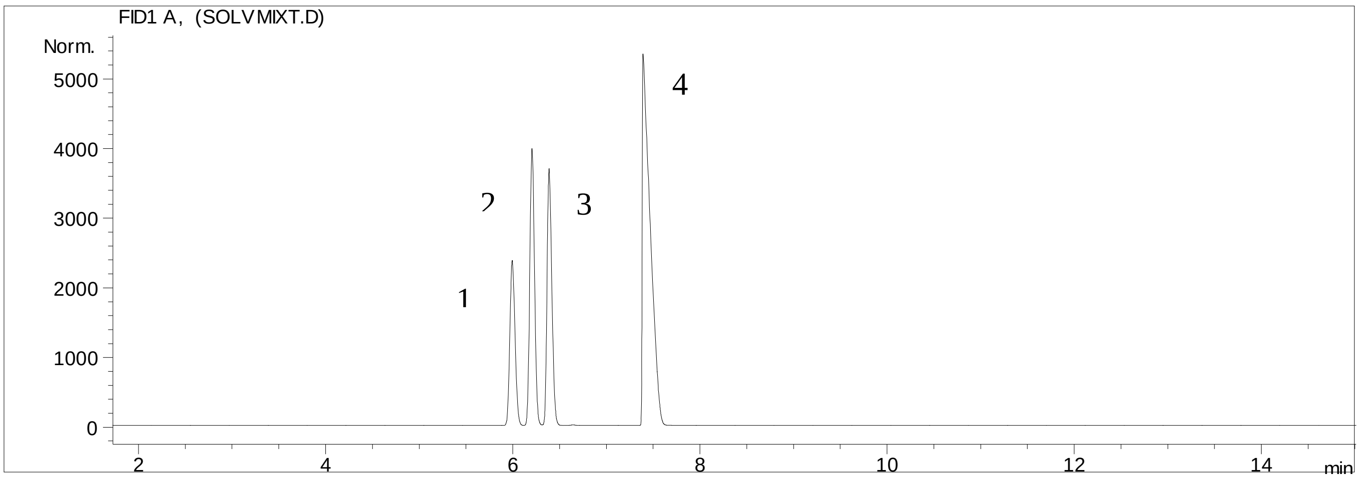
IX. Appendices

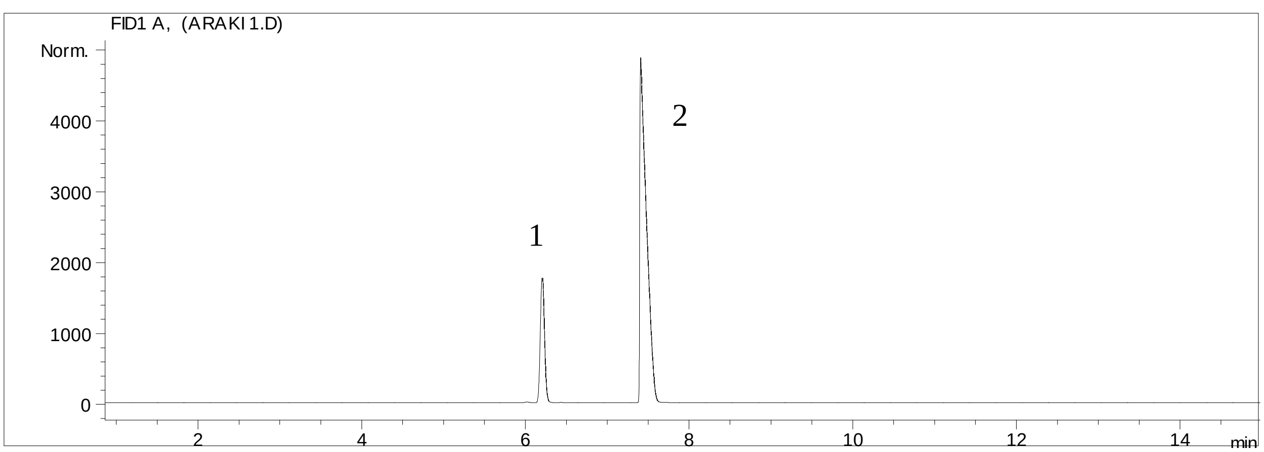
1. GC of Mixture of four volatile solvents

Peak Identified:
Peak 1: Methanol
Peak 2: Ethanol
Peak 3: Acetone
Peak 4: Chloroform



2. GC of Araki Dist.1 after dilution with $CHCl_3$

Peak Identified:
Peak 1: Ethanol (19%)
Peak 2: Chloroform (81%)

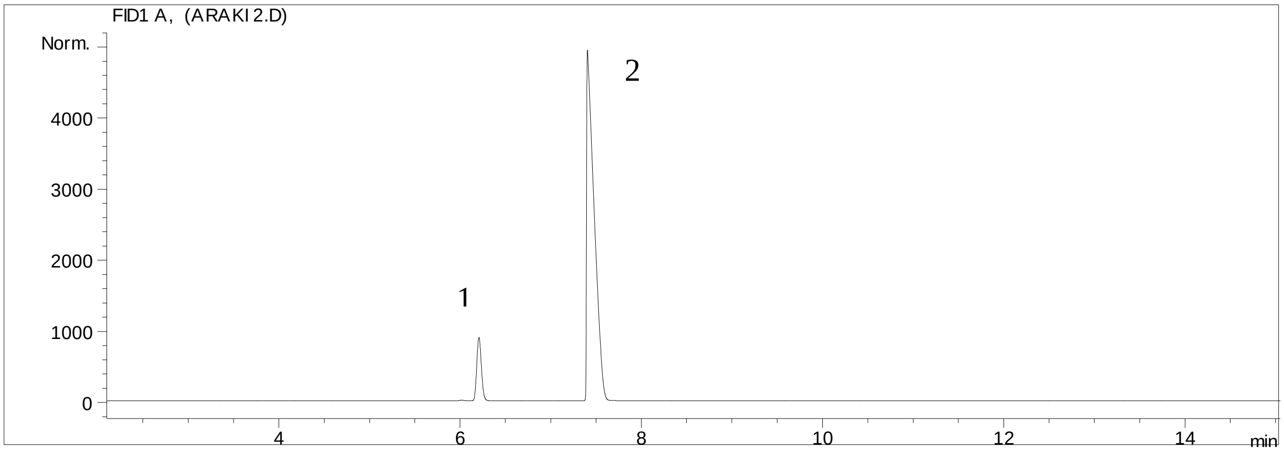


3. GC of Araki Dist.2 after dilution with $CHCl_3$

Peak Identified:

Peak 1: Ethanol (10%)

Peak 2: Chloroform (90%)

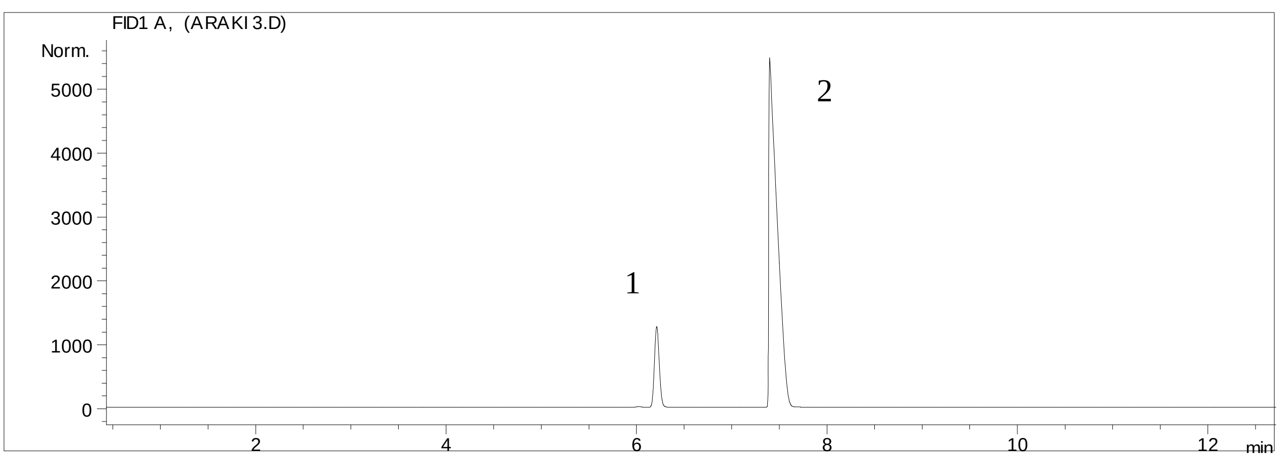


4. GC of Araki Dist.3 after dilution with $CHCl_3$

Peak Identified:

Peak 1: Ethanol (12%)

Peak 2: Chloroform (88%)

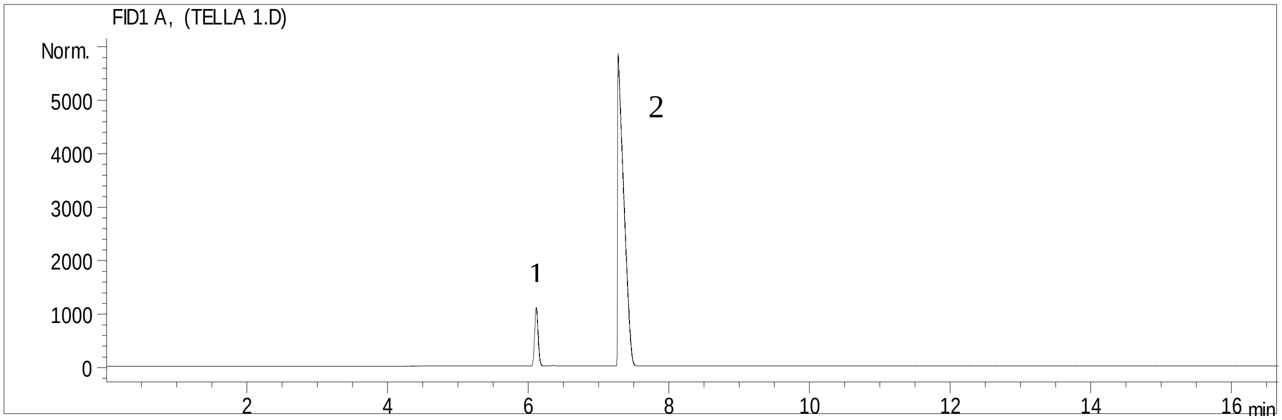


5. GC of **Tella 1 Diluted in $CHCl_3$**

Peak Identified:

Peak 1) Ethanol (10)

Peak 2) Chloroform (90%)

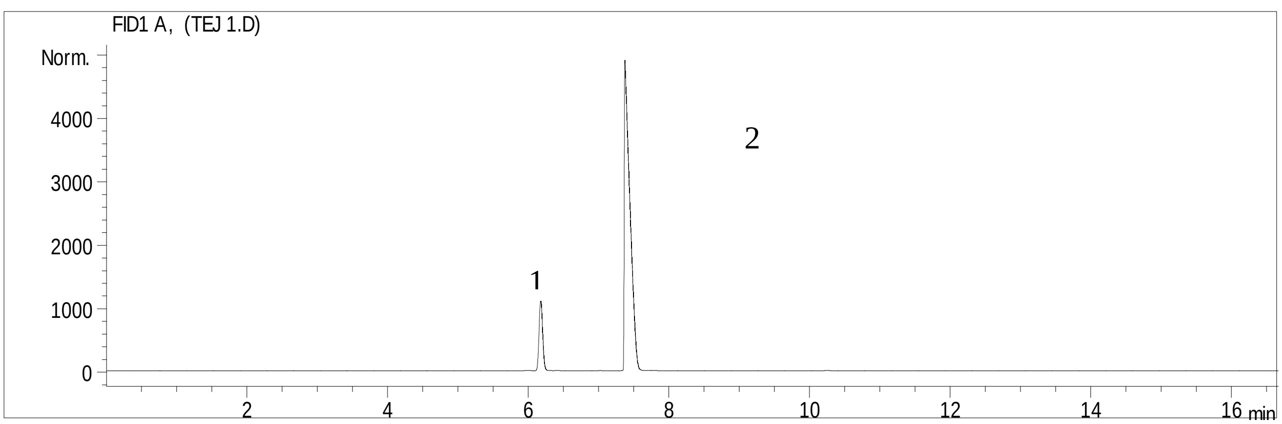


6. GC **TEJ 1 Diluted in $CHCl_3$**

Peak Identified:

Peak 1) Ethanol (13%)

Peak 2) CHCl2 (87%)



7. GC of Araki Dist.1 (neat)

Figure description:

Fig. 1
A)Araki _SD_Neat
B) Araki (MEOH added)
C) EtOH (Peak 2) and MeOH (Peak 1)

Fig. 2 Expanded Chromatogram of:
A)Araki _SD_Neat
B) Araki (MEOH added)
C) EtOH and MeOH

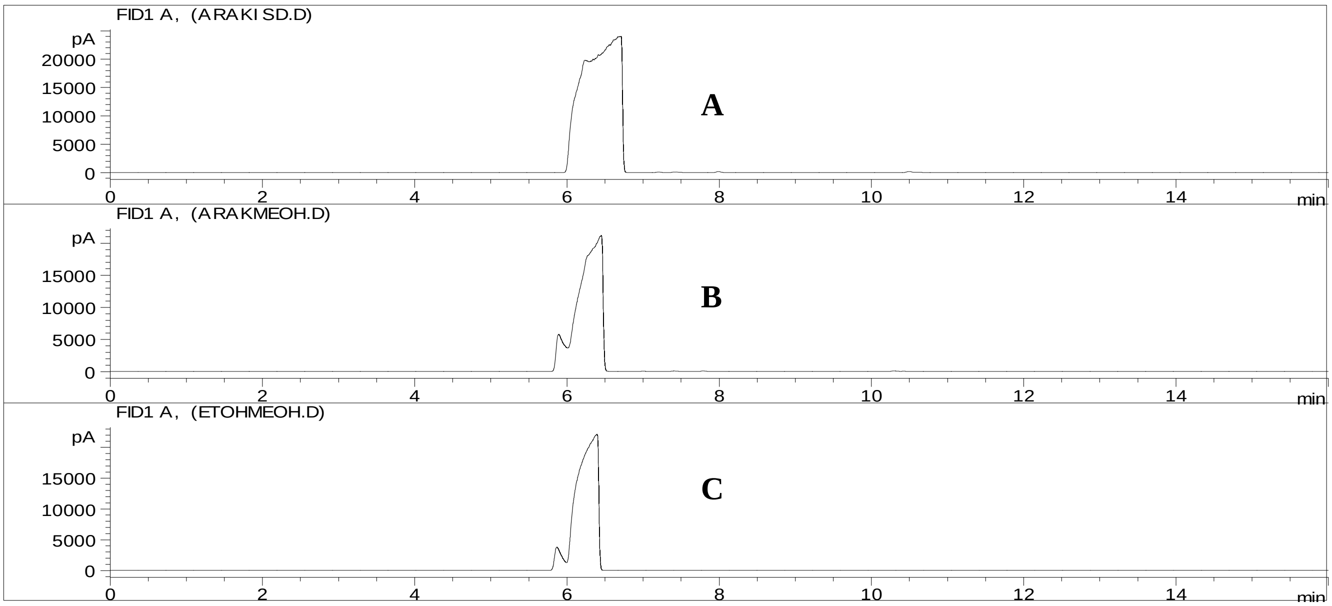


Fig.1

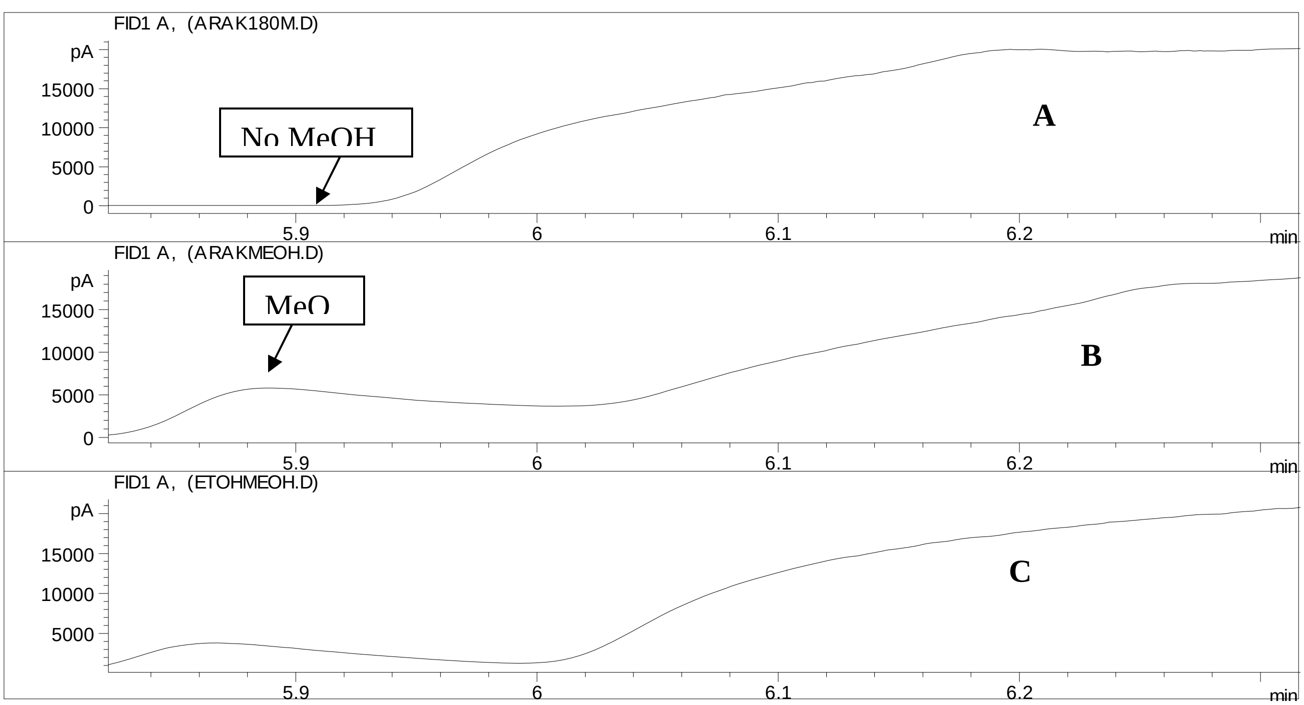


Fig. 2: Expanded Chromatogram