ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

DETERMINATION OF METHANOL, ETHANOL, SOLID, ACID CONTENT AND OTHER QUALITY PARAMETERS OF ETHIOPIAN TRADITIONAL FERMENTED, DISTILLED AND FACTORY PRODUCED ALCOHOLIC BEVERAGES

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October, 2018

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A THESIS SUBMITTED TO THE OFFICE OF GRADUATE PROGRAMS OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

By: Seferu Tadesse

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October, 2018

DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other University and that all the sources of materials used for this thesis has been duly acknowledged.

Name: Seferu Tadesse Signature ___________ Date__________

This thesis has been submitted for examination with our approval as university advisors.

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LIST OF ABBREVIATIONS

GC-MS	Gas Chromatography-mass Spectrometry
WHO	World Health Organization
CSA	Central Statistics Authority
ADH	Alcohol dehydrogenate
TS	Total solids
TDS	Total dissolved solids
TSS	Total suspended solids
ANOVA	Analysis of variance
FA	Fixed acid
VA	Volatile acid
TA	Total acid
EC	Electrical conductivity
WTO	World Trade Organization

ABSTRACT

The Ethiopian traditional fermented alcoholic beverages including (‘Tella’, ‘Tej’, ‘Shameta’,‘Bordie’ and ‘Korefie’), traditional distilled alcohols (‘Wheat Berkrakie Areki, ‘Wheat Sharata Areki’, ‘Dagusa Arefa Areki’, ‘Dagussa Sharata Areki’, ‘Dagim Areki’, ‘Gibto Areki’, ‘Wheat Arefa Areki’, ‘Mixed Areki’, ‘Koso Areki’ and ‘Yemar Areki’) and factory produced alcoholic beverages (‘Vodka Areki’, ‘Lomie Areki’, ‘Ananas Areki’, ‘Ouzo Areki’ and ‘Dry Gin Areki’) were collected from Addis Ababa, Ethiopia for the measurement of their ethanol, methanol, solid, salinity contents, acid value, pH value and electrical conductivity. The overall average values of pH value, ethanol% (v/v), acidic content (g/L), solid content (mg/L), conductivity (μS/cm) and salinity (%) found in all of the beverages studied were in the range of 4.6 - 7.5, 2.80 - 51.2, 0.02 - 105, 0.00 - 20328, 5.87- 8391 and 0.1 - 4.6, respectively. The methanol contents of the beverages were determined using GC-MS. The results showed that the level of methanol in some of the studied traditional and factory alcoholic beverages were found in the range between 0.867 - 0.979% (v/v). No methanol was observed in all of the analyzed traditional fermented beverages. Hence, contrary to the general assumptions, the levels of methanol observed in the analyzed drinks do not pose any health threat to the human. However, the normal alcohol health risk associated with high consumption remains a problem.

Key words: Alcoholic beverages, Methanol, Ethanol, Acids, GC-MS, Ethiopia

1. INTRODUCTION

Alcohol can be described as a psychoactive drug, which has extensive applications in both industries and households. Alcohol can be produced locally or factory produced in large or small quantities based on the available resources across the world. 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. The sources of raw materials for the production of alcohol can be obtained from fruits, cassava, palm wine, sugar cane, cereal crops, etc. The basic method for the production of alcohol is by fermentation or decomposition and simple distillation. 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. Dangerous chemical compositions such as lead and methanol present in alcohol pose a threat to human health. Ethanol and methanol have similar physicochemical properties and are present as by-products after distillation (Paine and Dayan, 200).

According to World Health Organization (WHO, 2014) 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, 2014). It is believed that methanol may be generated in alcoholic drinks during the fermentation process. 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 (Canaroglu and Yilmaztekin, 2011). The complications and chronic diseases or ill health such as blindness, dizziness, 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). Methanol is a chemical solvent widely used in products such as paints, gasoline and photocopying fluid. Methanol occurs naturally in food notably in fresh fruits, vegetables and their juices. It occurs as free methanol, methyl esters of fatty acids or methoxy groups on polysaccharides such as pectin from which it can be released by digestion. Pectin is broken down during digestion in the colon from where methanol can be absorbed. This means that the potential methanol intake from the diet is higher than analysis of the free methanol content of individual foodstuffs. The ingestion of as little as 10 mL of methanol can cause permanent blindness and 30 mL can be fatal. Methanol is metabolized by alcohol dehydrogenate that converts it to formaldehyde and finally to formic acid the metabolite responsible for its toxicity. Foliate is necessary to metabolize and excrete formic acid. Observed human oral lethal dosage of methanol via ingestion ranges from 15 to 250 g (Paine and Dayan, 200). 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. Alcoholic beverages have been widely consumed since prehistoric times by people around the world seeing use as a component of the standard diet, form hygienic or medical reasons or recreational purposes and for other reasons. The oldest alcoholic drinks were fermented beverages of relatively low alcoholic contents (Yohannes et al., 2013). 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 (Wang et al., 2013). 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 men. The method is inexpensive, easily acceptable and adaptable at household level in traditional communities. Fermentation processes enhance the nutritional quality of raw ingredient by improving the digestibility of nutrients and inactivating anti-nutritional factors. They also improve acceptability of the food by destroying undesirable flavors of the raw ingredients (Yohannes et al., 2013).

Fermentation is a widely practiced ancient technology and fermented beverages 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 produced 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 (Mulaw and Tesfaye, 2017). Ethanol is the only type of alcohol that can be consumed by human. 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 the 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 block outs, loss of control, etc (Oladeinde et al., 2002).

1.1 Statement of the problem

In assumption, the chemical properties of beverages home distilled Areki and factory produced distilled drinks can pose health threats due to their high alcoholic strength and undesirable additives. In Ethiopia, there is different traditional fermented, traditional distilled and factory produced alcohols. The ethanol, methanol, solids, salinity, conductivity and acidic content of all types of alcoholic beverages are not known. Therefore, this research focused on quantifying the ethanol, methanol, solids, salinity, conductivity and acidic content of in some fermented and distilled alcoholic drinks consumed in Ethiopia particularly in Addis Ababa. Even if, all analyzed parameters are factors for the quality of beverages methanol is very toxic and a critical to determine the quality of beverages than others. Several studies have found that the addition of pectolytic enzymes induces an increase of methanol levels in various fermentation products (Canaroglu and Yilmaztekin, 2011). Hence, it is believed that higher methanol content may be present in some alcoholic drinks produced by traditional distilleries or some alcohol brewing companies. In addition, some alcoholic beverage companies have used the natural ingredients extract from herbal using methanol as a solvent that the residual level of methanol might be present in these products (Kofi et al., 2017). Because of this, there could be a higher risk of methanol concentration in products. Therefore, for consumer protection to determine the content of methanol in alcoholic beverages is urgently required as increasing commercial exchanges of these alcoholic products between countries are expected to be more and more countries will join the World Trade Organization (WTO).

1.2 Objectives

1.2.1 General objective

The main objective of this study is to determine methanol, ethanol and acid content of locally and factory produced alcoholic beverages.

1.2.2 Specific objectives

- To determine the ethanol, solids, acids, pH, salinity and conductivity of alcoholic beverages.
- To determine the methanol content of alcoholic beverages by using GC - MS.
- To compare the ethanol, methanol, acids, solids, pH, salinity and conductivity content between different kinds of alcoholic beverages.
- To evaluate the influence of cereal type and areas in ethanol content of alcoholic beverages.

1.3 Significance of study

The outputs of this research work will be used to support and check the existing ethanol, methanol, acidic content and other quality parameters information on the Ethiopian traditional alcoholic beverages. It will be also used as a reference for future researches in various fields related to Ethiopian traditional alcoholic beverage samples and to assess influence of areas and types of cereals in ethanol content of traditional alcoholic beverages.

2. 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 (Wang et al., 2003). Fermented beverages vary considerably in type. Fermented beverages produced from cereals are usually referred to as beers while those produced from fruits are classified as wines (Canaroglu and Yilmaztekin, 2011). Indigenous fermented alcoholic beverages from different parts of the world are described). 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, 2006). Indigenous Ethiopian fermented beverages include Tej (Berza and Wolde, 2014), Tella (Sahle and Gashe, 1991), Bordie and Shameta (Debebe, 2006), Areki (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 (Tafere, 2015). Kenyan muratina and uragua are consumed largely at festivals and social gatherings. Palm wines (toddy's) 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 (Kofi et al., 2017). In West Africa in addition to their use as beverages, toddy's are also used as medicines for fever and other ailments by adding barks or stems of certain pants. It has often been observed that alcoholism is a more significant problem than all other forms of drug abuse combined. 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 (Ellison et al., 2001).

A report from Zambia as quoted by (Kebede et al., 2002) indicates that moulds such as *Mucor* could frequently be found on the fermenting source of pectinase, the enzyme that breaks down pectin to release methanol. Methanol shown to be the common contaminants of traditional alcoholic beverages in the studies carried out so far. According to (Canaroglu and Yilmaztekin, 2011) methanol is highly toxic and can cause blindness insanity and even death depending on the amount consumed. Fusel oil is a collective name of isopentyl alcohol, 2-methyl-1-butanol, isobutyl alcohol, propyl alcohol, esters and aldahydes. It is toxic and has been shown to cause cancer in experimental animals (Ellison et al., 2001). These alcohols are responsible for the severe headache and thirst associated with hangover and also account for taste and flavor of alcoholic drinks. From traditional fermented beverages in Ethiopia the most popular alcoholic beverages are Tej (honey wine), Tella (a malt beverage like beer), Shameta, Bordie and Areki (distilled liquor). These drinks are widely served on celebrations and at social gatherings. Traditional recipes are handed to new generations and are still used for food processing in many developing countries (Yohannes et al., 2013). The traditional 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. 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. 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.

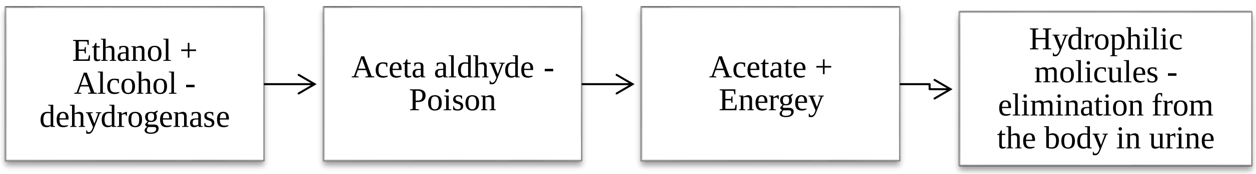

Figure 1. The metabolize process of ethanol in human body after drinks

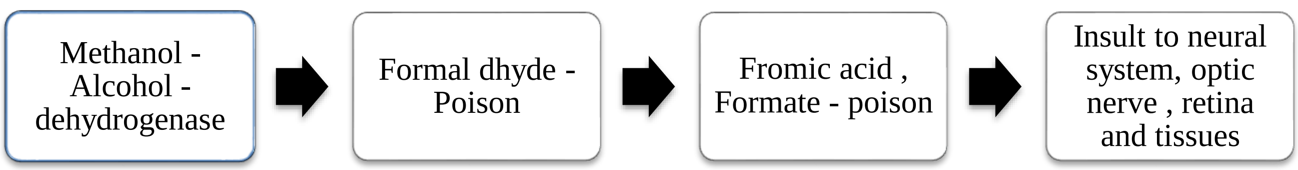

Figure 2. The metabolize of methanol in human body after drinks

Alcoholic beverages are generally divided into three different types, i.e. wine, beer and distilled spirits (Dudley, 2004). Beer and wine are produced by the fermentation of sugar or starches from cereal grains. The alcohol concentrations of these beverages are usually lower when compared to distilled spirits, which has higher alcohol content as it is produced by fermentation followed by distillation. Ethiopia is supplied with all these different types ranging from imported wine and liquors (distilled spirits like whisky, Vodka, Gin) to beers produced by local factories and non-commercial (traditional) drinks. Non-commercial (illicit) alcohol is defined as traditional alcohol drinks produced for home consumption or limited local trade. The very fact that illicit or non-commercial beverages are unrecorded makes it obviously difficult to assess their accurate alcohol contents or how much of this types of local drinks are being produced and consumed compared to legally sold alcohols. Yet they remain to be the most widely consumed alcohol, as they are inexpensive and easily accessible than factory produced beverages. For this reason factory produced drinks tend to be mainly consumed by people who can afford the more expensive price and by urban dwellers while locally produced and home-brewed alcoholic beverages are predominantly used in the rural areas and by people living in the urban areas who cannot afford factory made drinks. For instance young people (as young as 14 years old and university students) tend to consume local beverages like Areki and Tella as their main source of alcohol since these drinks are easily accessible to them and cannot afford other kinds of factory drinks (Keno and Keskis, 2015). These traditional or locally produced alcohols are also quite significant in economic terms. Many households in the country especially women engage in the production and sales of these beverages as their main source of income to support themselves and their families. Some of the popular locally produced and home-brewed beer, wine and distilled alcohol beverages are the following:

2.1 Tella

Tella is an Ethiopian home-brewed beer which differs from the others in some respects. First, it is brewed with barley or wheat, hops, or spices. Secondly, it has a smoky flavor due to the addition of bread darkened by baking and use of a fermentation vessel, which has been smoked by inversion over smoldering wood. Tella is not processed under government regulations hence the alcohol content varies but is usually around 3.84 to 6.48 (Tekluu et al., 2015). This drink is usually perceived as a harmless social drink and is usually prepared by women except in monasteries and church compounds. Tella is almost never sold in bars but in small houses "Tella bets". It is also the beverage of choice for family occasions and religious celebrations (mahebers). It is very popular and highly valued, as its production requires considerable skill and patience. It is by far the most commonly consumed alcoholic beverage in the country and assumed that over two million hectoliters of it is brewed annually in households and drinking houses in Addis Ababa (Debebe, 2006).

2.2 Shameta

Shameta 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. It is the local beer made among the Gurage ethnic group. Shameta 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 Shameta fermentation, although local Shameta 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 Shameta from a previous fermentation as well as from the ingredients and equipment. Ready to consume Shameta has a high microbial count made up of mostly lactic acid bacteria and yeast (Bacha et al., 2003). 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 (Bacha et al., 2003).

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. 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. According to Debebe (2006) during the preparation of Tej, the fermentation pot is seasoned by smoking over smoldering Gesho (*Rhamnus prenoides*) stems and olive wood. One part of honey mixed Areki with 2 to 5% (v/v) parts of water is placed in the pot covered with a cloth for 2 to 3 days to ferment after which wax and top scum is removed. Some portion of the must is boiled with washed and peeled Gesho (*Rhamnus prenoides*) and 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 (*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. Fermentation of Tej like other traditional 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 acidity produced during the fermentation can be hazardous to health if produced beyond acceptable levels. With the variable micro flora of such spontaneous fermentation, variability of the product is inevitable (Bahiru, 2001). The ethanol content of Tej is reported to range from 8.94% to 13.16 % (Yohannes et al., 2013) and 9.07% (Bahiru, 2001).

2.4 Bordie

Bordie is a traditional fermented beverage of Ethiopia a common meal replacement in Southern Ethiopia and some other parts of the country (Abegaz et al., 2002). According to the report of Yohannes., et al 2013) it is consumed while actively fermenting and has a short fermentation period usually overnight. On the average, a laborer consumes about three liters of Bordie per day. Bordie is prepared from 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. Tef is less frequently used for Bordie 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 Bordie preparation varies both within and between localities. Maize followed by Barley and Wheat was found to be the most common ingredient of Bordie both as malt and as un malted ingredient in southern Ethiopia where as Wheat has been reported to be the preferred un malted ingredient in Addis Ababa. As reported by (Abegaz et al., 2002). Some brewers in Hwassa and at Bedessa used both Finger millet and Sorghum as un malted ingredient whereas some at Gununo and Sodo zuria used Sorghum but not Finger millet. Sorghum was used as un malted ingredient but not as malt in the study areas. Finger millet was more frequently utilized for malt preparation than as un malted 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. Bordie is also used for medical and ritual purposes. Consumers believe that Bordie enhances lactation and mothers are encouraged to drink substantial amounts of it after giving birth. Bordie 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 (Abegaz et al., 2002).

2.5 Korefie

Korefie is the name of the local beer made in Begemder province among the Koumant ethnic group. Dehusked barley is left in water overnight after that roasted and milled. It is mixed Areki with water and dried gesho leaves, after that fermented in a clay container for two to three months. When the beverage is needed a small quantity of the mixture is taken more water is added and after a day's fermentation the beverage is ready for consumption (Getnet and Berhanu, 2016).

2.6 Areki

Areki or katikala is a home-distilled beverage with alcohol content ranging from 33.6 to 54.0% (v/v) ethanol. This beverage is more expensive than the other drinks and is popularly viewed as very strong and dangerous to consume. It is a colorless and 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 (Bekele and Chandravanshi, 2012). 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 factory produced alcohol (WHO, 2014). Traditional based on their way of process 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’. Based on their source of ingredients Areki have different varaties. The main ones are Dagussa Areki, Gibto Areki, Mar Areki, Wheat Areki and Koso Areki. Based on production process Dagussa Areki is two types Dagusa Arefa Areki and Dagussa Sharata Areki. Wheat Areki have also different types such as Wheat Berkerakie Areki, Wheat Sharata Areki and Wheat Arefa Areki. The basic way of preparation of this, different varieties of Areki are similar. Ground gesho leaves and water are kept for three to four days and after that a kita made of tef or other cereals and germinated Barley or Wheat is added. The mixture is allowed to ferment for five to six days and then distilled. In the villages, distillation is carried out with primitive equipment made of gourds and wood. The Areki can be redistilled and will then have higher alcohol content. The average alcohol content of Dagim Areki is around 51.0% (v/v) (Debebe et al., 2017). Areki is 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. Since the government has no control over production of locally brewed alcoholic drinks it is difficult to estimate the amount of alcohol production and consumption in Ethiopia.

2.6.1 Terra-Areki

Terra-Areki is a colorless and clear local alcoholic beverage, which is distilled from a fermentation product known as yeareki-tinsis. Based on the raw materials used there are many types of Terra-Areki, some of these are Yemar Areki, Wheat Berkrakie Areki, Wheat Arefa Areki, Wheat Sharata Areki, Koso Areki, Gibto Areki, Dagusa Sharata Areki and Dagusa Arefa Areki. According to the report of yeareki-tensis is prepared by mixing powdered gesho leaves and powdered bikil (1:2 ratios) 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 Dagussa *(Elusine coracann)* 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, with more water well mixed Areki, 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 average alcohol content of Terra-Areki was reported to be 40.0% (v/v) (Yohannes et al.; 2013) and varies between 35.0 – 45.0% (v/v) (Debebe, 2006).

2.6.2 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. The redistilled Areki will then have higher alcohol content. The average alcohol content of Dagim Areki is around 51% (v/v) (Debebe et al., 2016). It was also reported to have a mean value of 46.6% (v/v) ethanol content (Yohannes et al.; 2013). 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, 2014). According to the report of (Bahiru, 2001) 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 Areki culture of spontaneous fermentation is the rule in African food fermentation and the disadvantages of Mixed Areki 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 beverages. 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 (Tafere, 2015). 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.

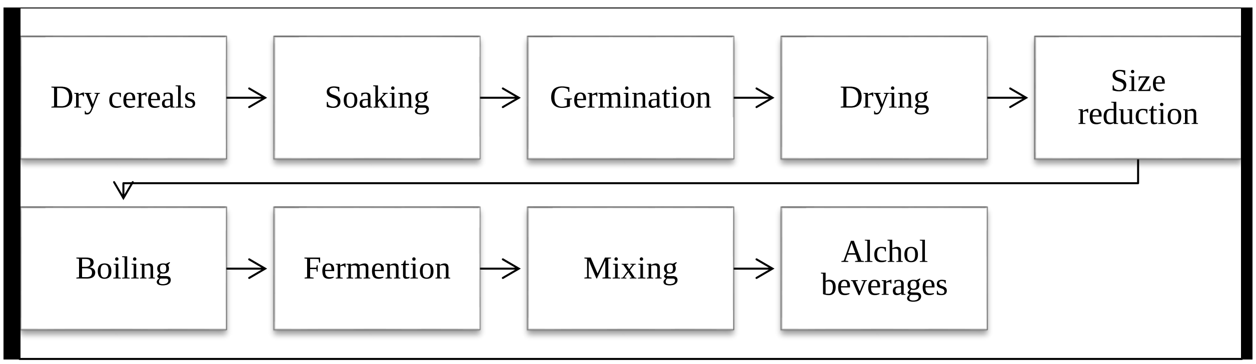

Figure 3. The process of making traditional alcoholic beverages from cereals

3. MATERIALS AND METHODS

3.1 Alcoholic beverage samples

For this study five types of traditional fermented (Tella, Tej, Shameta, Bordie, Korfie) and ten traditional distilled (Yemar Areki, Dagim Areki, Wheat Arefa Areki, Wheat Sharata Areki, Dagusa Arefa Areki, Dagussa Sharata Areki, Wheat Berkrakie Areki, Koso Areki, Mixed Areki and Gibto Areki) alcoholic beverages were collected from Addis Abaeba (Kality and Kotibe). Five most commonly factory produced alcoholic beverages (Lomie, Ananas, Ouzo, Dry gin and Vodka) were purchased from five different grocery. All the samples were collected based on universality of consumption from adolescents to the elderly and were widely available at both the local market and the household levels. From each sampling area, three samples each of 500 mL were collected from different vending houses, which were selected randomly to prepare a bulk sample to get representative sample. Thus, analyzing of the mixing of samples instead of analyzing single sample can reduces the variance and resource consumption. The collected alcoholic beverages were grouped as traditionally fermented, traditionally distilled and factory produced beverages. The raw materials used for the preparation of these beverages are differed from one another depending on the type of beverage. In general, the most widely used raw materials are cereals, fruits and selected herbs. A one single sample was prepared in the laboratory with equal volume ratios from the mixture of traditional distilled alcohols which includes Wheat Berkrakie, Wheat Sharta, Wheat Arfa and Dagim Arekies. This is because most of the women who sell different types of mixed Arekies. Such type of action is done intentionally to get more profit from it. For instance, Dagim Arekie which is good and high cost and Wheat Shrata and Wheat Berkrakie which were low quality and cheap. Triplicate analysis was performed for each sample. All the samples were collected using polyethylene plastic bottles and the bulk samples were kept in refrigerator at 4 ºC until the analysis was done.

3.2 Chemicals

Methanol (99.9%, Sigma-Aldrich, France), ethanol (99.9%, Fisher Scientific, UK), anhydrous ethyl acetate (99.8%) and sodium sulfate (99%) were utilized to prepare standard solutions. Sodium hydroxide pellets (98%), hydrochloric acid (37%) and phenolphthalein indicator (98%) were used for the determination of acidic content of alcohols. Distilled water was used for dilution of samples.

3.3 Instrument and apparatus

The apparatus used in this study are: beakers, burette, oven, measuring cylinders, pipettes, volumetric flasks, spatula, separator funnel, glass filter, what man filter paper (11 cm Diameter, China), orbital shaker (KS125 basic, Germany), centrifuge (Janetzki, model T32c, Olympus, Japan), electronic balance (SP 1500, USA). Whereas, fractional distillation set up, pH meter and Agilent GC 7890 coupled to a MS 5975 (Agilent Technologies, CA, USA) instruments were used in this study.

3.3.1 Alcoholic beverage sample preparation for GC-MS

The method was combined with a simple sample preparation procedure using sodium sulfate and anhydrous ethyl acetate. It is used only 1000 μL of alcoholic beverage sample to quantify all volatile components of samples. 1000 μL of alcoholic beverage sample mixed with 2000 μL anhydrous ethyl acetate in 10 mL test tubes. Approximately 100–150 mg of sodium sulfate salt was added to the mixture until saturation followed by intense mixing using a vortex mixer for 10 – 15 s. The samples were centrifuged for 2 min at 4000 r/min to separate the aqueous and non-aqueous layers. Approximately 200 μL of the upper non-aqueous phase was transferred to a 2 mL GC-MS vial for analysis (Pinu and Boas; 2017).

3.4 Determination of methanol content

3.4.1 Gas chromatography-mass spectrometry (GC-MS)

The methanol content of alcoholic beverages were analyzed using an Agilent GC 7890 coupled to a MS 5975 (Agilent Technologies, CA, USA) with a quadruple mass selective detector (electron impact ionization positive mode) operated at 70 eV. A Zebron ZB-1701 (Phenomenex) 30 m*250 μm (internal diameter)*0.15 μm (film thickness) with 5 m guard column was used for the analysis. 1 μL of sample was injected into the GC under split mode at a 100:1 split ratio under constant flow of 48.851 mL/min use helium gas as a carrier gas on column. The temperature of the inlet was kept at 180 ^{o}C. The GC oven temperature was initially held at 50 ^{o}C for 1 min and then raised to 200 ^{o}C at 40 ^{o}C/min. The total running time for this method was 4.75 min. The equilibration time was set to 2 min. The interface and quadruple temperatures were 230 ^{o}C and 150 ^{o}C, respectively. The MS detector was turned off between 2.03 min to 2.21 min to offload ethyl acetate peak. The MS was operated in scan mode with a mass range of 30 to 250 a.m.u. The ion (m/z) was used for identification and quantification of target compounds (Pinu and Boas, 2017).

3.5 Determination of pH values of alcoholic beverages

The pH measurements were carried out using a conventional pH glass electrode with analytical sensors and a pH meter Orion model SA 72. Before using it, a calibration routine was performed with pH 4, 7, and 10 buffers. Subsequently, the sensor was immersed in the beverages and the pH was measured in triplicate. The pH electrode was rinsed with abundant distilled water before and after each pH measurement. All experiments were performed at room temperature (Yücesoy, 2011).

3.6 Determination of acids in alcoholic beverages

Acid values was evaluated by titration with standardized solution of 0.1 N sodium hydroxide using phenolphthalein as indicator and the results were expressed as tartaric acid content (AOAC official methods (945.08, 950.15, 962.12,17th Ed, 2010). Preferred total acidity levels of beverages are 6 – 8 g/L (Jahagirdar et al., 2015). Volatile acidity is derived from the acids of the acetic series present in alcohols in the free state and combined as salts where as fixed acidity is derived from tartaric acid series. The content of total and fixed acids in terms of tartaric acid is calculated by using the formula:

$$\text{Total acid g tartaric acid/L} = \frac{0.00375 * 2 * \text{vol. of NaOH taken for titration} * 1000 * 2}{\text{The absolute alcohol percent by volume}}$$

(AOAC official methods (945.08, 950.15, 962.12,17th Ed, 2010)

$$\text{Fixed acid g tartaric acid/L} = \frac{0.00375 * 2 * \text{vol. of NaOH taken for titration} * 1000 * 2}{\text{The absolute alcohol percent by volume}}$$

(AOAC official methods (945.08, 950.15, 962.12,17th Ed, 2010)

Volatile acidity is determined from the difference of total acidity and fixed acidity

$$\text{Volatile acidity}(\frac{g}{L}) = \text{Total acidity}(\frac{g}{L}) - \text{fixed acidity}(\frac{g}{L})$$

3.7 Determination of solids in alcoholic beverages

The dissolved solids of alcohols were determined by Conductivity Meter (EC-215R Hanna Instrument, ITALY). Electrode was immersed in samples and recorded the reading display on it. Each sample was analyzed three times. The desirable limit for TDS is between 500 mg/L and 2,000 mg/L (Jahagirdar et al., 2015; Shyam et al., 2015). Total solids were determined according to the method set by AOAC Official Method (920.47, 940.09, 950.27, 17th Ed, 2010)**.** Primarily dry empty dish with 105 ^{o}C for 30 min. Take out from oven and put in dissector until it reaches to room temperature. Then weight empty dish and add 100 ml samples on it. Put the sample in oven with 105 ^{o}C for 3 hours. Take out the sample from oven and put immediately in dissector. Wait until it reaches to room temperature. Weight the sample and recorded it. Total solids in alcohols were calculated by:

$$\text{Total solids}\ (\frac{w}{w}) = \text{Weight of dry sample and dish} - \text{weight of empty dry dish}$$

Suspended solids were determined by taken the difference from total solids and total dissolved solids.

$$\text{Suspended solids}(\frac{w}{v}) = \text{Total solids} - \text{Total dissolved solids}$$

3.8 Determination of electrical conductivity and salinity of alcoholic beverages

Conductivity measurements were carried out with an Orion 4 Stars conduct meter. The procedure consisted in calibrating the instrument with 1413 μS and 12.9 mS/cm standards and subsequently the sensor was immersed in beverage sample and the conductivity was measured in triplicate. The electrode was rinsed with abundant water before and after each immersion. All experiments were performed at room temperature (Alejandra et al.; 2016).

3.9 Determination of ethanol content in alcoholic beverages

The ethanol level of collected samples was determined by fractional distillation method (AOAC Official Method (920.47, 942.06, 945.07, 982.10, 983.12 17th Ed); ASBC official method Beer-4; 27 CFR part 30, 2010). The method is applied for all types of traditional and factory produced alcohols. The method is verified by running spiked absolute alcohol which ethanol content is 99.99% for each type of samples and by calculating its recovery. Based on this the recovery was found in the range 91 - 107%, which is acceptable and indicates that the method is functional and applicable for the analysis of selected samples. The procedure for the determination was as follows: taken 100 mL samples with measuring flask and added it in to a separating funnel. Sufficient powdered sodium chloride was added to saturate the liquid. To this, 50 mL of petroleum ether was added in order to extract ethanol and other volatile components. The solubility of ethanol and petroleum ether is very high as compared to the solubility of water and ethanol. The mixture of ethanol and petroleum ether makes a separate layer from aqueous phase. After 15 min the layer of water was removed from the separator funnel while the layer of petroleum ether was poured in to the distillation flask. The layer of petroleum ether was washed with 20 mL of saturated sodium chloride solution two times and added the washing solutions in to distillation flask. The mixture of ethanol and petroleum ether was set to distillation process. The distillation was done by fractional distillation. The final temperature of the ethanol to completely evaporate was identified by running absolute ethanol. This is done by using absolute ethanol five times. Then with similar sample preparation, process the distillation was performed. Finally, it was waited until the recovery absolute ethanol was 100%. Based on the five repeated data the final temperature was 72 ^{o}C. At this temperature, ethanol was completely evaporated and collected in the receiver. Whatever the time allowed it did not evaporate other volatile components whose boiling point is more than ethanol. Therefore, the optimum boiling point of ethanol was 72 ^{o}c. By setting this temperature, the ethanol level of the alcohols was determined according to the following formula.

$$\text{Ethanol level\%}\ \left(\frac{\text{v}}{\text{v}}\right) = \frac{\text{Sample volume} + \text{Volume of petrolium ether}}{\text{Volume of distillate sample} + \text{Volume of petrolium ether}} * 100$$

4. RESULTS AND DISCUSSION

Several analytical parameters of various beverage samples produced in Ethiopia were measured in this study. Having different crop varieties as the raw material, distillation process and alcohol content lead to different chemical properties (Yücesoy, 2011). All the results with regard to the determination of pH, electrical conductivity, salinity, solid, acid, methanol and ethanol content of beverage samples are listed with mean values and standard deviations in Table 2 - 5 and in Table 9. In the study Mixed Areki sample were the mixture of Wheat Berkrakie, Wheat Sharta, Wheat Arfa and Dagim Arekies.

4.1 Methanol content in alcoholic beverages

4.1.1 Calibration curve of methanol in ethyl acetate for GC-MS

Methanol concentration of (1.00 - 100 mg/L) was measured using GC-MS. Then the peak area of chromatogram versus concentration (Figure 4) was constructed to validate the GC-MS concentration of methanol in terms of linearity, sensitivity, precision and for calibration purpose to determine the methanol content of various beverage samples. From the calibration curve (Figure 4), the calibration equation was y = 189x + 12930, R^2 = 0.999, where y is peak area of chromatogram, x is concentration of methanol and R is the linear regression coefficient. The calibration curve was observed for a wide concentration range, which is convenient for the determination of methanol in various beverages. Peak area and retention time of analyzed beverage samples was known from the data of GC- MS chromatogram. By inserting peak area of chromatogram in linear equation of calibration curve, the concentrations of analyzed alcoholic beverages were determined.

4.1.1 Standard methanol solutions for GC-MS

The concentration of methanol used for preparing standard samples was 99.9% (v/v). By using the density of methanol, it can be convert to mg/L. Methanol 99.9% (v/v) means 99.9 mL methanol is present in 100 mL solution. The density of methanol is 0.791 g/mL by using the formula of density:

$$\text{Density methanol} = \frac{\text{mass methanol}}{\text{volume methanol}}$$

$$\text{Mass of methanol} = \text{density of methanol} \times \text{volume of methanol}$$

$$\text{Mass of methanol} = \frac{0.791\ \text{g}}{\text{mL}} \times 99.9\ \text{mL}$$

From this 79.02 gram of methanol is present in 100 mL of solution. This is equivalent to 790200 mg/L. By using, the formula $C1V1 = C2V2$ methanol stock solution (500 mg/L) was prepared by dissolving 316 µL of absolute methanol (99.9% or 790200 mg/L) with 500 mL distilled water. Then 1, 10, 30, 60 and 100 mg/L methanol working solutions were prepared from stock solution in 25 mL volumetric flasks.

Table 1. Concentration and peak area of methanol standard solutions from the GC-MS.

Concentration (mg/L)	1	10	30	60	100
Area of the peak (mAU)	12929553	13218461	13409071	14099584	14797899

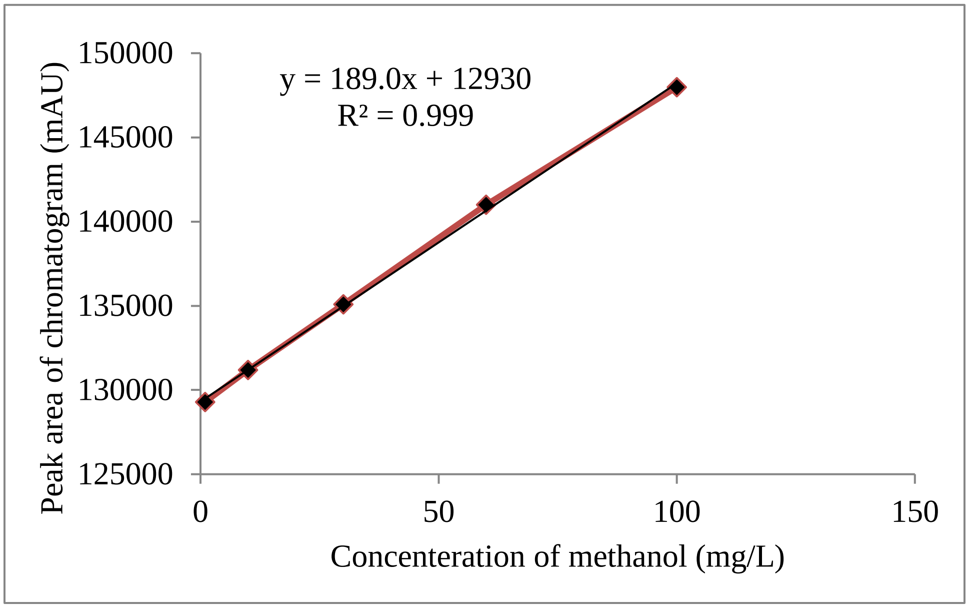

Figure 4. Calibration curve of methanol standard for GC-MS

Table 2. Methanol content of traditional fermented, distilled and factory produced alcoholic beverages

No	Beverage types	Peak area	Methanol concentration (mg/L)
1	Dry gin	1301031	6852 ± 31.8
2	Daussa Shrata	1301618	6872 ± 53.2
3	Ouzo	1321681	6930 ± 50.5
4	Mixed	1325426	6944 ± 57.9
5	Wheat Sharata	1330075	6936 ± 29.1
6	Vodka	1341229	7035 ± 58.6
7	Koso	1385390	7244 ± 75.4
8	Dagim	1431145	7525 ± 81.7
9	Yemar	1433920	7504 ± 29.2
10	Wheat Arefa	1458666	7667± 86.3
11	Dagussa Arefa	1301031	7654 ± 35.2
12	Wheat Berkrakie	1301618	7735 ± 45
13	Gibto	-	ND
14	Lomie	-	ND

15	Ananas	-	ND
16	Tej	-	ND
17	Shameta	-	ND
18	Bordie	-	ND
19	Tella	-	ND
20	Korfie	-	ND

Table 2 shows the variations in the concentrations of methanol of the various beverage samples analyzed. Methanol concentration of the beverage samples ranged from "not detectable" to 7735 mg/L. From Table 2, it was also observed that all traditional beverages (Tej, Tella, Korefie and Bordie), one traditional distilled beverages (Gibto Areki) and two traditional fermented beverages (Lome and Ananas Areki) recorded no content of methanol concentration where as nine of traditional distilled beverages (Dry Gin, Dagussa Sharata, Ouzo, Mixed, Wheat Sharata, Vodka, Koso, Dagim, Yemar, Wheat Arefa, Dagussa Arefa and Wheat Berkrakie) and three of factory produced beverages (Dry gin, Ouzo and Vodka) showed traces of methanol at different concentrations. Among the samples that tested positive for methanol content Dry Gin (factory produced areki) recorded the lowest amount of methanol concentration (6852 mg/L) while Wheat Berkrakie Areki (traditional distilled Areki) showed the highest methanol concentration (7735 mg/L). The results show that low content of methanol were recorded in the factory-produced beverages as compared to the traditional distilled beverages. The oral lethal dose of methanol between 0.3–1 g/kg (20 to 60 g or 25–75 mL/person in a 60 kg adult) (Tulashie eta al.; 2017). A 7735 mg/L methanol level as seen in the analysis implies that 7.74 g methanol may be realized in 1 L of the alcoholic drink (Wheat Berkrakie) and hence possesses no potential health threat when consumed. However, it has a health problem in 60 kg adult when drinks continuously 3.2 L- 9.7 L Wheat Berkrakie Areki.

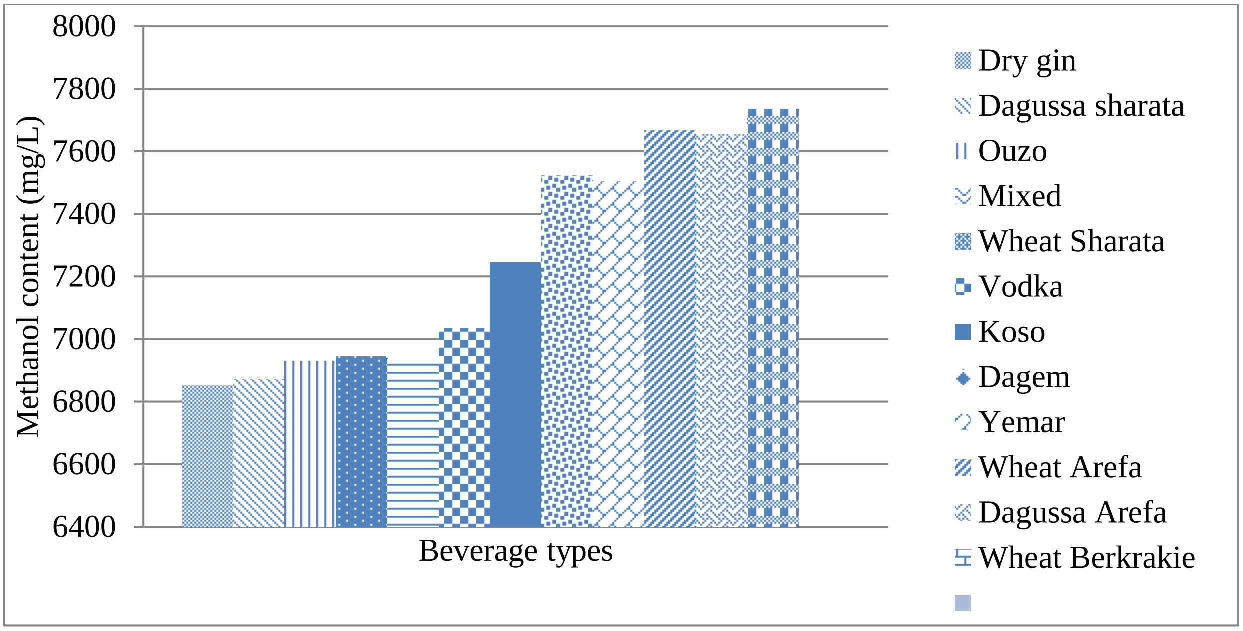

Figure 5. Methanol content of traditional fermented, traditional distilled and factory produced alcoholic beverages.

As it can be seen in Figure 5, generally the content of methanol across the beverage types increased in order of Drygin < Daussa Shrata < Ouzo < Wheat Sharata < Mixed < Vodka < Koso < Dagim < Yemar < Wheat Arefa < Dagussa Arefa < Wheat Berkrakie.

4.2 Determination of pH, conductivity, salinity, acidity and solid contents in factory produced alcoholic beverages

Table 3. Quality parameters in factory produced alcoholic beverages.

Quality parameters	Factory produced alcoholic beverages				
	Vodka	Lomie	Ananas	Ouzo	Dry gin
pH	7.00 ± 0.01	5.50 ± 0.01	6.50 ± 0.01	7.50 ± 0.04	5.90 ± 0.02
Conductivity (µS/cm)	5.87 ± 0.06	121 ± 0.3	61.5 ± 0.2	135 ± 0.15	9.60 ± 0.2
Salinity (%)	0.000	0.100 ± 0.1	0.000	0.000	0.100 ± 0.01
TDS (mg/L)	3.04 ± 0.05	57.1 ± 0.1	29.0 ± 0.8	64.0 ± 0.1	5.03 ± 0.08
TSS (mg/L)	65.0 ±1	11667 ±3.5	10152 ±3.1	256 ±1	1125 ±0.6
TS (mg/L)	67.7 ± 0.6	11723 ± 2.5	10183 ± 2.5	322 ± 2.9	1132 ± 2
Fixed acidity (g/L)	0.024 ± 0.005	0.069 ± 0.006	0.076 ± 0.006	0.023 ± 0.005	0.043 ± 0.005
Volatile acidity (g/L)	0.000	0.100	0.000	0.000	0.000
Total acidity (g/L)	0.024 ± 0.005	0.156 ± 0.006	0.041± 0.006	0.032 ± 0.005	0.043 ± 0.005

* = Values are mean ± SD of triplicate readings of triplicate analysis

Total acid results of factory produced beverage samples ranged from 0.024 - 0.156 g tartaric acid/L. The mean total acid values of Vodka, Lomie, Ananas, Ouzo and Dry gin beverages were 0.024, 0.156, 0.041, 0.032 and 0.043 g tartaric acid/L, respectively. Lomie Areki had the highest and Vodka had lowest total acid value from factory produced beverages. Volatile acid results of factory produced traditional beverage samples ranged from 0.000 - 0.100 g tartaric acid/L. The mean volatile acid values of Vodka, Lomie, Ananas, Ouzo and Dry gin beverages were 0.000, 0.100, 0.000, 0.000 and 0.000 g tartaric acid/L, respectively. Lomie Areki had the highest volatile acid value from factory produced beverages. No factory produced beverage samples had volatile acids except Lomie areki had 0.100 mg tartaric acid/L. Fixed acid results of factory produced beverage samples ranged from 0.023 - 0.076 g tartaric acid/L. The mean fixed acid values of Vodka, Lomie, Ananas, Ouzo and Dry gin were 0.024, 0.069, 0.076, 0.023 and 0.043 g tartaric acid/L, respectively. Ananas Areki had the highest whereas, Ouzo Areki had lowest fixed acid value from factory produced beverages. Preferred total acidity levels of beverages are 6 – 8 g tartaric acid/L (Jahagirdar et al., 2015). Therefore, total acid content of below 6 g tartaric acid/L as seen in all of the analyzed drinks (Vodka, Lomie, Ananas, Ouzo and Dry gin) implies that below 6 g of tartaric acid is contained in 1 L of the alcoholic drink and may all the same had low quality contents.

Total dissolved solid results of factory produced beverage samples ranged from 3.04 - 64.0 mg/L. The mean TDS values of Vodka, Lomie, Ananas, Ouzo and Dry gin beverages were 3.04, 57.1, 29.0, 64.0 and 5.03 mg/L, respectively. TDS values of Ouzo Areki had the highest and Vodka had lowest TDS value from factory produced beverages. Total suspended solid results of factory produced beverage samples ranged from 65.0 - 11667 mg/L. The mean TSS values of Vodka, Lomie, Ananas, Ouzo and Dry beverages were 65, 11667, 10152, 256 and 1125 mg/L, respectively. Lomie Areki had the highest and Vodka had lowest TSS value from factory produced beverages. Total solid results of factory produced beverage samples ranged from 67.7 - 11723 mg/L. The mean total solid values of Vodka, Lomie, Ananas, Ouzo and Dry gin beverages were 67.7, 11723, 10183, 322 and 1132 mg/L, respectively. Lomie Areki had the highest whereas, Vodka had lowest total solid value factory produced beverages. Preferred solid content of beverages are 500 – 2000 mg/L (Jahagirdar et al., 2015). Therefore, total solid content of 2000 mg/L and above as seen in two factory produced beverages (Lomie and Ananas) implies that at least 2000 mg of solid is contained in 1 L of the alcoholic drink and may all the same cause higher health risks due to excess solids when consumed excessively or without care. The solid contents of Vodka, Ouzo and Dry gin had within the standard range as set by Drinking water standards as per BIS 10500 (Jahagirdar et al., 2015).

The mean pH values of Vodka, Lomie, Ananas, Ouzo and Dry gin beverages were 7.00, 5.49, 6.48, 7.50 and 5.85 respectively. pH value of Ouzo Areki were highest and Lomie Areki had lowest than other factory produced beverages. Preferred pH levels of beverages are are 3.5 – 4.0 (Rajković et al.; 2007). All the pH of factory produced beverages (Vodka, Lomie, Ananas, Ouzo and Dry gin) pH was out of the range of standards. Among the studied factory produced beverages, Lomie Areki had better pH levels than Vodka, Ananas, Ouzo and Dry gin. Whereas, Ouzo had low quality as compared to Vodka, Lomie, Ananas and Dry gin.

Electrical conductivity results of factory produced beverage samples ranged from 5.87 - 135 μS cm^{-1}. The mean electrical conductivity values of Vodka, Lomie, Ananas, Ouzo and Dry gin beverages were 5.87, 121, 61.5, 135 and 9.60 μS/cm, respectively. Electrical conductivity of

Ouzo Areki were highest whereas, Vodka Areki had lowest than other factory produced beverages. Among the studied factory produced beverages, Vodka had better quality electrical conductivity than Lomie, Ananas, Ouzo and Dry gin. Whereas, Ouzo had low quality as compared to Lomie, Ananas and Dry gin. The mean salinity values of Lomie and Ouzo Areki, were 0.100%. In result no salinity was observed in three factory produced beverages (Vodka, Ananas and Ouzo) while 0.100% of salinity were recorded in Dry Gin and Lomie Areki's.

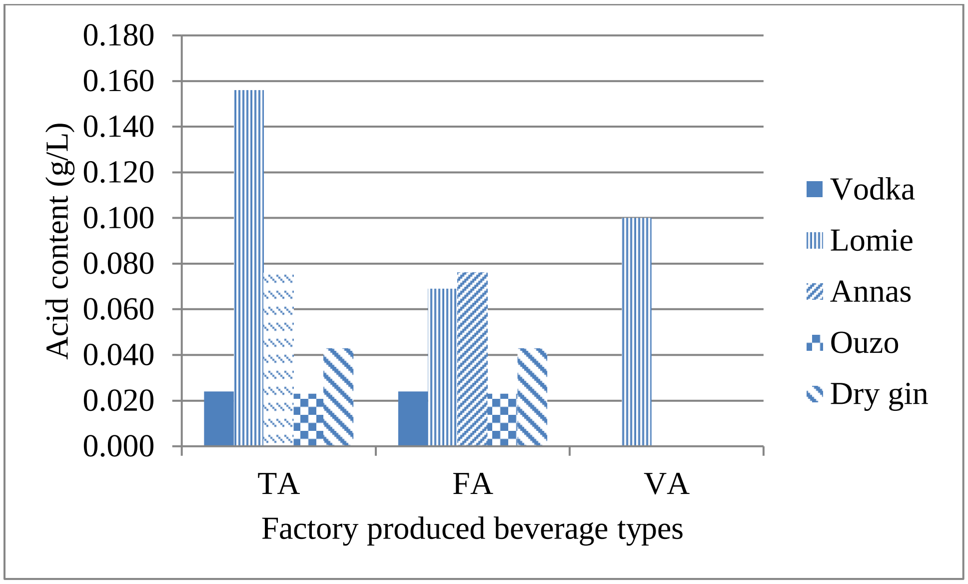

Figure 6. The comparison of total acidity, fixed acidity and volatile acidity contents in g/L between factories produced alcoholic beverages.

As it can be seen in figure 6, generally the content of total acid across the beverage types decreased in order of Lomie > Dry gin > Ananas > Ouzo > Vodka Whereas, the volatile acid of Lomie > Vodka = Ananas = Dry gin = Ouzo and fixed acid of Ananas > Lomie > Dry gin > Vodka > Ouzo.

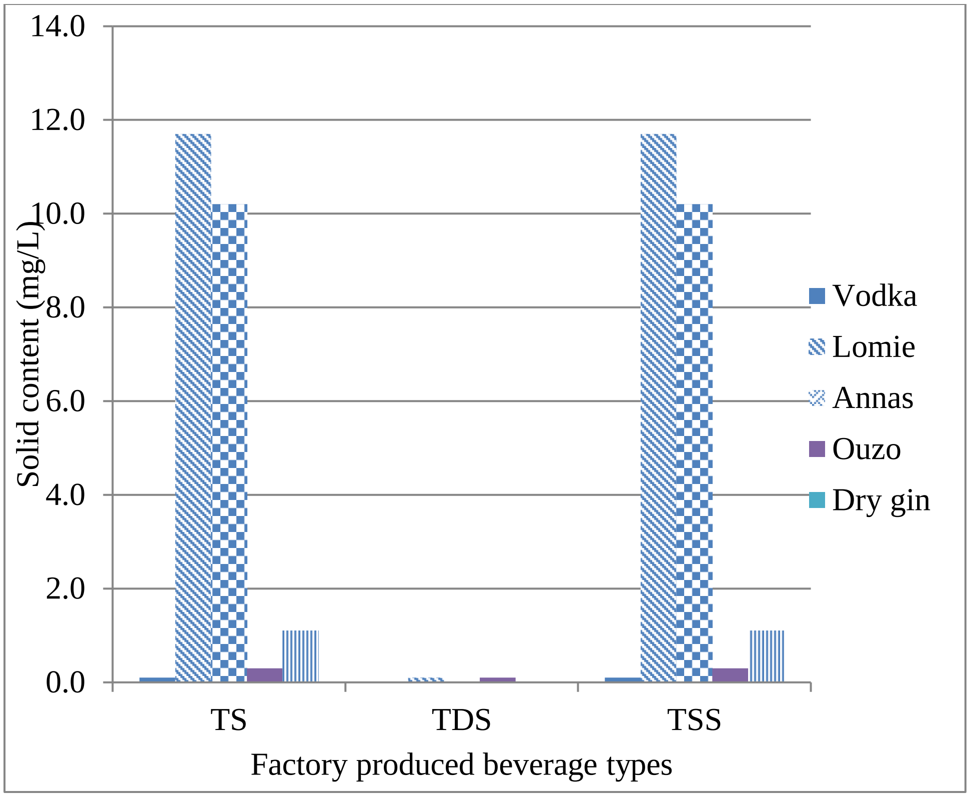

Figure 7. The comparison of TS, TDS and TSS contents in mg/L between factories produced alcoholic beverages.

As it can be seen in Figure 7, generally the content of total solid across the factory produced beverage types decreased in order of Lomie > Ananas > Dy gin > Ouzo > Vodka Whereas, the TDS of Ouzo > Lomie > Ananas > Dry gin > Vodka and TSS of Lomie > Ananas > Dry gin > Ouzo > Vodka.

4.3 Determination of pH, conductivity, salinity, acidity and solid contents in traditional fermented alcoholic beverages

Table 4. Quality parameters in traditional fermented alcoholic beverages.

Quality parameters	Traditional fermented alcoholic beverages				
	Tej	Shameta	Bordie	Tella	Korefie
pH	3.60 ± 0.01	3.80 ± 0.01	3.50 ± 0.01	3.80 ± 0.01	3.70 ± 0.01
Conductivity (μS/cm)	811 ± 1.00	8391 ± 1	7139 ± 1	2359 ± 1.5	3199 ± 1
Salinity (%)	0.400 ± 0.001	4.60 ± 0.001	3.90 ± 0.002	1.20 ± 0.001	1.70 ± 0.001
TDS (mg/L)	387 ± 1	4520 ± 1	3830 ± 0.58	1180 ± 0.58	1610 ± 0.58
TSS (mg/L)	4676 ± 2.5	11274 ± 1	16498 ± 1	540 ± 0.58	5680 ± 0.58
TS (mg/L)	5063 ± 2.5	15795 ± 2	20328 ± 1.5	1721 ± 1.5	7291 ± 1
Fixed acidity (g/L)	6.86 ± 0.06	55.9 ± 0.34	9.27 ± 0.27	2.86 ± 0.03	4.34 ± 0.05
Volatile acidity (g/L)	9.81 ± 0.03	49.4 ± 0.28	70.2 ± 0.14	30.6 ± 0.33	85.2 ± 0.4
Total acidity (g/L)	16.7 ± 0.03	105 ± 0.06	79.5 ± 0.410	33.5 ± 0.30	89.5 ± 0.35

[*] = Values are mean ± SD of triplicate readings of triplicate analysis

Total acid results of traditional fermented beverage samples ranged from 16.7 - 105 g tartaric acid/L. The mean total acid values of Tej, Shameta, Bordie, Tella and Korfie beverages were 16.7, 105, 79.5, 33.5 and 89.5 g tartaric acid/L, respectively. Thus, Shameta had the highest total acid value whereas; Tej had the lowest total acid value. The results of volatile acid content (in terms of tartaric acid) of traditional fermented beverage samples ranged from 9.81 - 85.2 g tartaric acid/L. The mean volatile acid values of Tej, Shameta, Bordie, Tella and Korfie beverages were 9.81, 49.4, 70.2, 30.6 and 85.2 g tartaric acid/L, respectively. Korfie had the highest and Tej had lowest total acid value beverage. Fixed acid results of traditional fermented beverage samples ranged from 2.86 - 55.9 g tartaric acid/L. The mean fixed acid values of Tej, Shameta, Bordie, Tella and Korfie beverages were 6.86, 55.9, 9.27, 2.86 and 4.34 g tartaric acid/L, respectively. Shameta had the highest and Tella had lowest fixed acid value from factory produced beverages. The acid content plays an important role in alcoholic beverages for the preservation and sensory characteristics of beverages. Preferred total acidity levels of beverages are 4 – 8 g tartaric acid/L (Rajković et al., 2007). Therefore, total acid content of 8 g tartaric acid/L and above as seen in all traditional fermented beverages (Tej, Shameta, Bordie, Tella and Korfie) implies that at least 8 g of tartaric acid is contained in 1 L of the alcoholic drink and may all the same cause higher health risks due to excess acids when consumed excessively or without care. Among the studied traditional, fermented beverages Tej had better quality than from Shameta, Bordie, Tella and Korfie. Whereas, Shameta had low quality as compared to Tej, Bordie, Tella and Korfie. However, all acid values of the traditional fermented beverages (Tej, Shameta, Bordie, Tella and Korfie) were found to be above the standard values (Rajković et al., 2007).

Total dissolved solid results of traditional fermented beverage samples ranged from 387 - 4520 mg/L. The mean TDS values of Tej, Shameta, Bordie, Tella and Korfie beverages were 387, 4520, 3830, 1180 and 1610 mg/L, respectively. TDS values of Shameta had the highest and Tej had lowest TDS value from traditional fermented beverages. Total suspended solid results of traditional fermented beverage samples ranged from 540 - 16498 mg/L. The mean TSS values of Tej, Shameta, Bordie, Tella and Korfie beverages were 4676, 11274, 16498, 540 and 5680 mg/L, respectively. TSS values of Bordie had the highest and Tella had lowest TS value from traditional fermented beverages. Total solid of alcohols is the combination of suspended and dissolved solids. Total solid results of traditional fermented beverage samples ranged from 1721 - 20328 mg/L, respectively. The mean TS values of Tej, Shameta, Bordie, Tella and Korfie beverages were 5063, 15795, 20328, 1721 and 7291 mg/L, respectively. TS values of Bordie had the highest and Tella had lowest TS value from traditional fermented beverages. Preferred solid content of beverages are 500 – 2000 mg/L (Jahagirdar et al., 2015). Therefore, total solid content of 2000 mg/L and above as seen in four traditional fermented beverages (Tej, Shameta, Bordie and Korfie) implies that at least 2000 mg of solid is contained in 1 L of the alcoholic drink and may all the same cause higher health risks due to excess solids when consumed excessively or without care. Among the studied traditional, fermented beverages, Tella had better quality than from Shameta, Bordie, Tej and Korfie. Whereas, Bordie had low quality as compared to Tej, Shameta,Tella and Korfie. However, all solid values of the traditional fermented beverages except tella (Tej, Shameta, Bordie, and Korfie) were found to be above the values set by Drinking water standards as per BIS 10500 (Jahagirdar et al., 2015).

pH values of traditional fermented beverage samples ranged from 3.47 - 3.80. The mean pH values of Tej, Shameta, Bordie, Tella and Korfie beverages were 3.80, 3.59, 3.80, 3.47, 3.80 and 3.73, respectively. pH value of Shameta were highest and Bordie had lowest pH values from traditional beverages. Preferred pH levels of beverages are are 3.5 – 4.0 (Rajković et al.; 2007).

All the pH of traditional beverages (Tej, Shameta, Bordie, Tella and Korfie) pH was in range of standards. Hence, not all beverages can cause higher health risks due to pH when consumed.

Electrical conductivity results of traditional fermented beverage samples ranged from 811 - 8391 μS cm^{-1}. Measuring electrical conductivity was indicated as a practical method to identify fraud beverages (Lachenmeir et al.; 2008). The conductivity of beverages was very stable between replicate measurements of the same type. However, it shows large differences between different beverage types. The mean electrical conductivity values of Tej, Shameta, Bordie, Tella and Korfie beverages were 811, 8391, 7139, 2359 and 3199 μS/cm, respectively. Electrical conductivity of Shameta were highest and Tej had lowest electrical conductivity value from traditional fermented beverages. High electrical conductivity values can mean beverage that tastes bad or is too salty. Among the studied traditional, fermented beverages, Tej had better quality electrical conductivity than from Shameta, Bordie, Tella and Korfie. Whereas, Shameta had low quality as compared to Tej, Bordie, Tella and Korfie. Salts can affect the quality of water used for preparation of beverages. They also have a critical influence on Humans health. The mean salinity values of Tej, Shameta, Bordie, Tella and Korefie were 0.4%, 4.6%, 3.9%, 1.2% and 1.7%, respectively. Salinity of Shameta were highest whereas, Bordie had lowest than other fermented beverages.

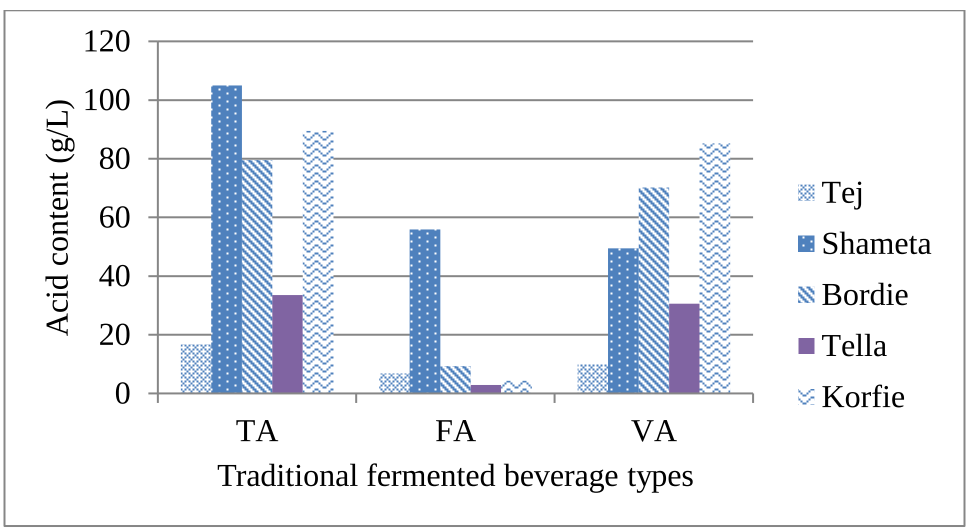

Figure 8. The comparison of total acidity, fixed acidity and volatile acidity contents in g/L between traditional fermented alcoholic beverages.

As it can be seen in Figure 8, generally the content of total acid across the beverage types decreased in order of Shameta > Korfie > Bordie > Tella > Tej whereas, the volatile acid of Korefie > Bordie > Shameta >Tella >Tej and fixed acid of Shameta > Bordie > Tej > Korefie >Tella.

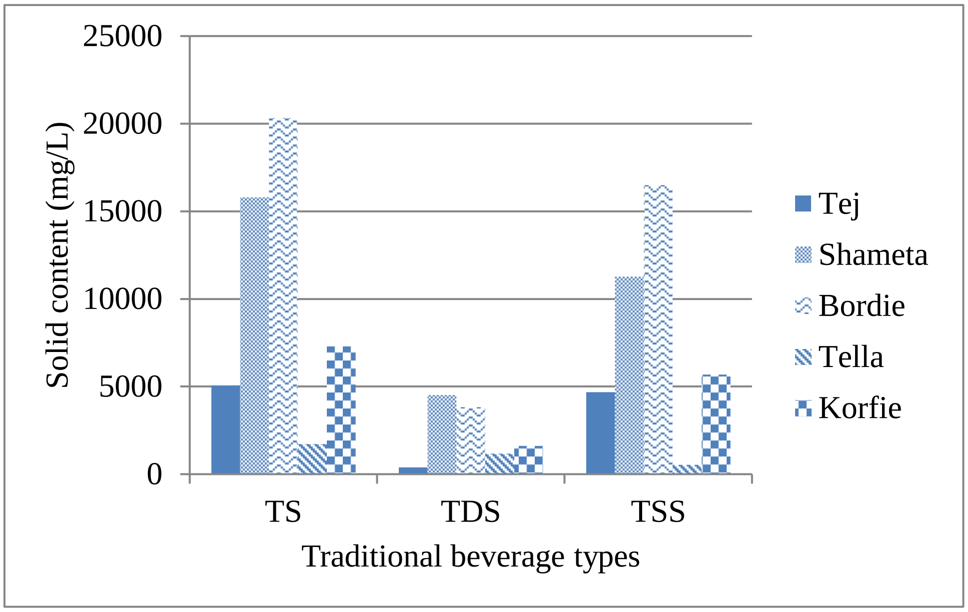

Figure 9. The comparison of TS, TDS and TSS contents in g/L between traditionally fermented alcoholic beverages.

As it can be seen in Figure 9, generally the content of total solid across the traditional fermented beverage types decreased in order of Bordie > Shameta > Korefie > Tej > Tella Whereas, the TDS of Shameta > Bordie > Korefie >Tella >Tej and TSS of Bordie > Shameta > Korefie > Tej > Tella.

4.4 Determination of pH, conductivity, salinity, acidity and solid contents in traditional distilled alcoholic beverages

Table 5. Quality parameters in traditional distilled alcoholic beverages.

Beverage types	Quality parameters in different traditional distilled alcoholic beverages								
	pH	Conductivity (μS/cm)	Salinity (%)	TDS (mg/L)	TSS (mg/L)	TS (mg/L)	Fixed acidity (g/L)	Volatile acidity (g/L)	Total acidity (g/L)
Yemar	4.60 ± 0.01	116 ± 1	0.100 ± 0.001	56.0 ± 0.1	7156 ± 2.08	7211 ± 1.2	0.163 ± 0.005	0.188 ± 0.01	0.350 ± 0.005
Gibto	4.40 ± 0.01	48.0 ± 0.5	0.000	21.9 ± 0.1	699 ± 1	720 ± 1.5	0.146 ± 0.004	0.205 ± 0.008	0.351 ± 0.004
Dagim	4.20 ± 0.01	19.0 ± 0.25	0.000	9.03 ± 0.1	1.00 ± 0.03	10.0 ± 0.2	0.018 ± 0.004	0.160 ± 0.006	0.178 ± 0.002
Wheat Arefa Areki	4.00 ± 0.01	24.0 ± 0.1	0.000	10.0 ± 0.1	0.000	10.0 ± 0.3	0.039 ± 0.004	0.214 ± 0.013	0.253 ± 0.009
Wheat sharata	4.00 ± 0.01	24.0 ± 0.15	0.000	0.000	0.000	0.000	0.026 ± 0.005	0.311 ± 0.007	0.337 ± 0.002
Dagussa Arefa	4.10 ± 0.01	26.0 ± 0.2	0.000	9.87 ± 0.2	0.000	10.0 ± 0.3	0.027 ± 0.004	0.177 ± 0.012	0.204 ± 0.016
Dagusa Sharata	4.10 ± 0.01	19.0 ± 0.21	0.000	100 ± 0.1	0.000	10.0 ± 0.3	0.023 ± 0.005	0.234 ± 0.003	0.257 ± 0.002
Wheat berkrakie	4.30 ± 0.01	25.0 ± 0.25	0.000	0.000	0.000	0.000	0.032 ± 0.005	0.283 ± 0.01	0.315 ± 0.003
Mixed Areki	4.10 ± 0.01	23.0 ± 0.15	0.000	11.2 ± 0.2	0.000	11.0 ± 0.4	0.114 ± 0.005	0.055 ± 0.00	0.169 ± 0.005
koso	3.80 ± 0.01	38.0 ± 0.12	0.000	17.8 ± 0.3	233 ± 1.53	249 ± 2.1	0.063 ± 0.002	0.067 ± 0.00	0.130 ± 0.004

[*] = Values are mean ± SD of triplicate readings of triplicate analysis

Total acid results of traditional distilled beverage samples ranged from 0.130 - 0.35 g tartaric acid/L. The mean total acid values of Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso beverages were 0.350, 0.351, 0.178, 0.253, 0.337, 0.204, 0.257, 0.315, 0.169 and 0.130 g tartaric acid/L, respectively. Gibto Areki had highest whereas, Koso Areki had lowest total acid value compared to Yemar, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie and Mixed traditional distilled beverages. Volatile acid results of traditional distilled beverage samples ranged from 0.05 - 0.310 g tartaric acid/L. The mean volatile acid values of Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso beverages were 0.188, 0.205, 0.160, 0.214, 0.311, 0.177, 0.234, 0.283, 0.055 and 0.067 g tartaric acid/L, respectively. Wheat Sharata Areki had highest and Mixed Areki had lowest volatile acid content compared to Yemar, Gibto, Dagim, Wheat Arefa, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie and Koso traditional distilled beverages. Fixed acid results of traditional distilled beverage samples ranged from 0.018 - 0.146 g tartaric acid/L. The mean fixed acid values of Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso beverages were 0.163, 0.146, 0.018, 0.039, 0.026, 0.027, 0.023, 0.032, 0.114, 0.063 g tartaric acid/L, respectively. Yemar Areki had highest whereas, Dagim Areki had lowest fixed acid value compared to Gibto, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso traditional distilled beverages. Preferred total acidity levels of beverages are 6 – 8 g tartaric acid/L (Jahagirdar et al., 2015). All acid content of analyzed drinks (Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso) were below the standard value set by (Jahagirdar et al., 2015).

Total dissolved solid results of traditional distilled beverage samples ranged from 0.000 - 100 mg/L. The mean TDS values of Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso beverages were 56.0, 21.9, 9.03, 10.0, 0.000, 9.87, 100, 0.000, 11.0 and 17.8 mg/L, respectively. TDS values of Dagussa Sharata Areki had highest whereas, Wheat Sharata and Wheat Berkarkie beverages had lowest compared to Yemar, Gibto, Dagim, Wheat Arefa, Dagussa Arefa, Mixed and Koso traditional distilled beverages. No dissolved solids were present in Wheat Sharata and Wheat Berkarkie beverages. Total suspended solid results of traditional distilled beverage samples ranged from 0.000 - 7156 mg/L, respectively. The mean TSS values of Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso beverages were 7156, 699, 1.00, 0.000, 0.000, 0.000, 0.000, 0.000, 0.000 and 233 mg/L, respectively. TSS values of Yemar Areki had highest whereas, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie and Mixed beverages had lowest TSS value compared to Gibto, Dagim and Koso traditional distilled beverages From the result of traditional distilled beverages no TSS was observed in Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie and Mixed beverages, where as Gibto Areki, Yemar Areki, Koso Areki and Dagim Areki had low content of suspended solids. Total solid results of traditional distilled beverage samples ranged from 0.000 - 7211mg/L. The mean TS values of Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso beverages were 7211, 720, 10.0, 10.0, 0.000, 10.0, 10.0, 0.000, 11.0 and 249 mg/L, respectively. TS values of Yemar Areki had highest whereas, Wheat Sharata and Wheat berkrakie had lowest TS value compared to Gibto, Dagim, Wheat Arefa, Dagussa Arefa, Dagussa Sharata, Mixed and Koso traditional distilled beverages. Preferred solid content of beverages are 500 – 2000 mg/L(Jahagirdar et al., 2015). Therefore, total solid content of 2000 mg/L and above as seen in one traditional distilled beverage (Yemar Areki) implies that at least 2000 mg of solid is contained in 1 L of the alcoholic drink and may all the same cause health risks due to excess solids when consumed excessively or without care. The solid contents of Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso had within the standard range as set by Drinking water standards as per BIS 10500 (Jahagirdar et al., 2015).

The mean pH values of Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso beverages were 4.60, 4.40, 4.20, 4.00, 4.00, 4.10, 4.10, 4.30, 4.10, 3.80 respectively. pH value of Yemar Areki were highest and Koso Areki had lowest pH value from traditional distilled beverages. Electrical conductivity results of traditional distilled beverage samples ranged from 19-116 μS cm^{-1}. The mean electrical conductivity values of Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso beverages were 116, 48.0, 19.0, 24.0, 24.0, 26.0, 19.0, 25.0, 23.0, 38.0 μS/cm, respectively. Electrical conductivity of Yemar Arekies were highest whereas, Dagim and Dagussa Sharata beverages had lowest electrical conductivity value from traditional distilled beverages. Among the studied factory produced beverages, Dagem and Dagussa Sharata had best electrical conductivity quality than Yemar, Gibto, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Wheat Berkrakie, Mixed and Koso. Whereas, Yemar areki had low quality as compared to Gibto, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Wheat Berkrakie, Mixed and Koso. The mean salinity values of Yemar Areki was 0.1%. In result no salinity was observed in nine traditional distilled beverages (Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed and Koso) while 0.100% of salinity were recorded in Yemar Areki.

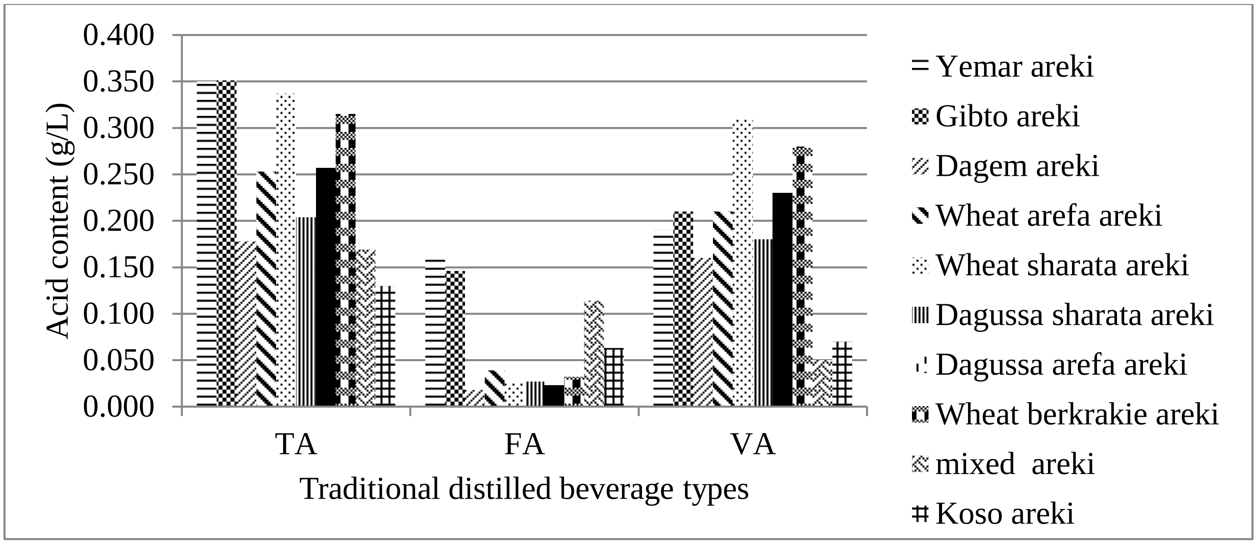

Figure 10. The comparison of total, fixed and volatile acidity contents in g/L between traditional distilled alcoholic beverages.

As it can be seen in figure 10, generally the content of total acid across the traditional distilled beverage types decreased in order of Gibto > Yemar > Wheat sharata > Wheat Berkrakie > Dagussa Sharata > Wheat Arefa > Dagussa Arefa > Dagim > Mixed > Koso Whereas, the volatile acid of Wheat Sharata > Wheat Berkrakie > Dagussa Shrata > Wheat Arefa > Gibto > Yemar > Dagussa Arefa > Dagim > Koso > Mixed and fixed acid of Yemar > Gibto > Mixed > Koso > Wheat Arefa > Wheat Berkrakie > Dagussa Arefa > Wheat Sharata > Dagussa Sharata > Dagim.

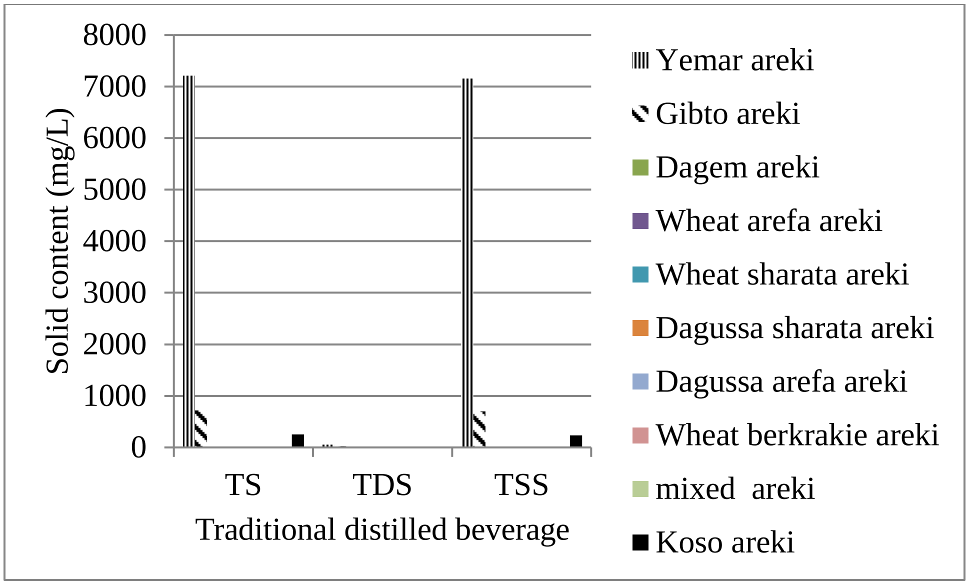

Figure 11. The comparison of TS, TDS and TSS contents in g/L between traditional distilled alcoholic beverages.

As it can be seen in figure11, generally the content of total solid across the traditional distilled beverage types decreased in order of Yemar > Gibto > Koso > Mixed > Dagim = Wheat Aarefa = Dagussa Arefa > Wheat Sharata = Wheat Berkrakie. Whereas, the total dissolved solid of Dagusa Sharata > Yemar > Gibto > Koso > Mixed > Wheat Arefa > Dagussa Arefa > Dagim > Wheat Berkrakie = Wheat Sharata and total suspended solid of Yemar > Gibto > Koso > Dagim > Wheat Shrata = Dagussa Arefa = Dagussa Sharata = Wheat Berkrakie.

4.5 Comparison of acid, solid, pH, conductivity and salinity contents across traditional distilled, fermented and factory produced alcoholic beverages.

Total acid values of Shameta and Korfie were highest compared to all beverages where as Vodka Areki had the lowest total acid value of 0.024 g tartaric acid/L. Fermented beverages have highest total acid values than distilled traditional and factory produced beverages. Volatile acid values of Korfie were highest compared to others where as Vodka, Ananas, Ouzo, Dry Gin Areki had the lowest volatile acid value of 0.000 g tartaric acid/L. Fermented beverages have highest volatile acid values than distilled traditional and factory produced beverages. Fixed acid values of Shameta were highest compared to all other beverages where as Dagim, Ouzo and Vodka Arekies had the lowest fixed acid value of 0.018 g tartaric acid/L, 0.023 g tartaric acid/L and 0.024 g tartaric acid/L respectively. Traditional fermented alcoholic beverages had high contents of total, fixed and volatile acidity than traditional distilled and factory produced alcoholic beverages. The acid amount in fermented and distilled beverages can vary in wide ranges due to crop variety, harvesting time of crops, climatic conditions during harvesting, type of the soil, phyto-sanitary condition of crops, way of malt processing, conditions which alcohol fermentation was done, beverage storage time, volatile compounds and additives (Rajković, 2007).

TDS values of Shameta and Bordie were highest compared to others where as Wheat Berkrakie and Wheat sharata had the lowest TDS value of 0.000 mg/L. Fermented beverages had highest TDS values than distilled traditional and factory produced beverages. From the results, traditional fermented beverages had significantly high content dissolved solids than distilled traditional and factory produced beverages. The highest TSS was observed in Shameta, Bordie and Lomie Areki and the lowest in Wheat Berkrakie, Wheat Sharata, Wheat Arefa, Dagussa Arefa, Dagussa Sharata and Mixed Areki. The content of suspended solids present in distilled traditional alcohols were lowest than traditional fermented and factory produced beverages. TS values of Shameta and Bordie were highest compared to others where as Wheat Berkrakie and Wheat sharata had the lowest TS value of 0.000 mg/L. Fermented beverages have highest TS values than distilled traditional and factory produced beverages. The solid content in fermented and distilled beverages can vary in wide ranges due to the difference in sources raw materials (crop varieties), process conditions, volatile compounds, additives, initial TSS as well as the difference in ferment ability behavior of the beverages (Yücesoy, 2011).

Fermented beverages have lowest pH values than distilled traditional and factory produced beverages. Differences in the raw materials (crop varieties), process conditions, volatile compounds, storage time and additives can cause the differences among the pH values. Electrical conductivity of Shameta and Bordie were highest compared to others and Vodka had the lowest conductivity value of 5.87 $\mu S\ cm^{-1}$. Conductivity of fermented beverages were very high than distilled beverages. Differences in the raw materials (crop varieties), process conditions, volatile compounds, storage time and additives can cause the differences among the electrical conductivity values (Lachenmeier et al.; 2008). Compared to distilled fermented beverages had highest salinity value. Salinity of Shameta and Bordie were highest compared to others.

Fermented beverages of Tella, Shameta, Korefie and Bordie had a high content of salinity because in the fermentation process no distillation is performed.

4.6 Ethanol content of alcoholic beverages

Recovery test

The recovery test was done for all the samples since all the samples had different matrices and the percentage recoveries were calculated by spiking known concentration of ethanol into the traditional alcoholic beverage samples. The efficiency of the fractional distillation method used for the determination of ethanol in alcoholic beverages was checked by adding known concentration of ethanol to the 50 mL different kind of alcoholic beverage sample. The amount of ethanol spiked was 20% to 30% the amount of ethanol determined in samples (Bekele and Chandravanshi, 2012).The same procedure for spiked samples as that of sample was followed. The different known spiked concentration of ethanol was prepared from absolute ethanol, its concentration was 99.99%. Each recovery test was performed in triplicates.

Table 6. Recovery test results for ethanol determination in samples of the traditional fermented alcoholic beverages.

Type of beverage	Concentration of ethanol in un spiked beverage sample% (v/v)	Amount of ethanol added% (v/v)	Concentration of ethanol in spiked beverage sample% (v/v)	Recovery% (v/v)
Tella	5.20	1.04	6.12 ± 0.10	98.0 ± 8
	5.20	1.30	6.37 ± 0.15	101.0± 3
	5.20	1.56	6.62 ± 0.25	103 ± 4
Shameta	3.20	0.64	3.76 ± 3	100 ± 5
	3.20	0.80	3.92 ± 3	99.0 ± 4
	3.20	0.96	4.08 ± 5	101 ± 3
Bordie	3.00	0.60	3.53 ± 8	103 ± 3
	3.00	0.75	3.68 ± 1	98.0 ± 3
	3.00	0.90	3.82 ± 1	105 ± 5
	6.00	1.20	7.06 ± 7	101 ± 8
Korfie	6.00	1.50	7.35 ± 9	105 ± 1
	6.00	1.80	7.64 ± 1	98.0 ± 1
Tej	12.0	2.40	14.1 ± 9	99.0 ± 7
	12.0	3.00	14.7 ± 9	100 ± 9
	12.0	3.60	15.6 ± 1	101 ± 1

Table 7. Recovery test results of ethanol determination in samples of the traditional distilled alcoholic beverages.

Type of beverage	Concentration of ethanol in un spiked beverage sample% (v/v)	Amount of ethanol added% (v/v)	Concentration of ethanol in spiked beverage sample% (v/v)	Recovery% (v/v)
	38.0	7.60	45.1 ± 0.01	99 ± 7
Yemar Areki	38.0	9.50	50.4 ± 0.01	106 ± 4
	38.0	11.4	52.9 ± 0.01	107 ± 2
	43.0	8.60	53.2 ± 0.05	103 ± 7
Gibto Areki	43.0	10.8	54.8 ± 0.03	102 ± 7
	43.0	12.9	54.8 ± 0.02	98.0 ± 2
	54.0	10.8	67.4 ± 0.15	104 ± 3
Dagim Areki	54.0	13.5	72.2 ± 0.15	107 ± 6
	54.0	16.2	67.4 ± 0.06	96.0 ± 3
	44.0	8.80	50.7 ± 0.04	96.0 ± 8
Wheat Aarfa Areki	44.0	11.0	51.2 ± 0.05	93.0 ± 3
	44.0	13.2	53.8 ± 0.04	94.0 ± 2
	36.0	7.20	43.2 ± 0.06	100 ± 1
Wheat Sharata Areki	36.0	9.00	47.3 ± 0.04	105 ± 2
	36.0	10.8	44.5 ± 0.02	95.0 ± 1
	48.0	9.60	58.8 ± 0.25	102 ± 1
Dagusa Arfa Areki	48.0	12.0	57.6 ± 0.05	96.0 ± 5
	48.0	14.4	61.8 ± 0.25	99.0 ± 1
	40.0	8.00	45.1 ± 0.15	94.0 ± 8
Dagusa Sharata Areki	40.0	10.0	46.5 ± 0.15	93.0 ± 4
	40.0	12.0	49.9 ± 0.06	96.0 ± 2
	42.6	8.52	50.1 ± 0.12	98.0 ± 6
Wheat Berkrakie Areki	42.6	10.7	50.1 ± 0.20	94.0 ± 2
	42.6	12.8	60.4 ± 0.15	109 ± 6
	39.5	7.90	51.2 ± 0.31	108 ± 3
Mixed Areki	39.5	9.88	52.3 ± 0.10	106 ± 4
	39.5	11.9	48.8 ± 0.15	95.0 ± 9
	46.0	9.20	50.2 ± 0.004	91.0 ± 3
Koso Areki	46.0	11.5	52.9 ± 0.01	92.0 ± 3
	46.0	13.8	59.2 ± 0.01	99.0 ± 5

Table 8. Recovery test results of ethanol determination in samples of the factory produced alcoholic beverages.

Type of beverage	Concentration of ethanol in un spiked beverage sample% (v/v)	Amount of ethanol added% (v/v)	Concentration of ethanol in spiked beverage sample% (v/v)	Recovery% (v/v)
Vodka Areki	40.0	8.00	46.8 ± 0.15	97.5 ± 0.10
	40.0	10.0	51.2 ± 0.10	102 ± 0.09
	40.0	12.0	50.9 ± 0.31	97.9 ± 0.04
Lomie Areki	30.0	6.00	39.3 ± 0.15	109 ± 0.02
	30.0	7.50	39.6 ± 0.20	105 ± 0.03
	30.0	9.00	36.8± 0.12	94.4 ± 0.01
Ananas Areki	32.0	6.40	41.7 ± 0.25	108 ± 0.03
	32.0	8.00	39.0 ± 0.05	97.6 ± 0.04
	32.0	9.60	40.3 ± 0.05	96.9 ± 0.17
Ouzo Areki	40.0	8.00	46.8 ± 0.25	97.5 ± 0.06
	40.0	10.0	51.2 ± 0.04	102 ± 0.02
	40.0	12.0	50.9 ± 0.04	97.9 ± 0.02
Dry gin Areki	40.0	8.00	52.4 ± 0.05	107 ± 0.02
	40.0	10.0	52.8 ± 0.06	105 ± 0.05
	40.0	12.0	51.4 ± 0.04	97.6 ± 0.01

The percentage of the recovery was between 91 and 107% (Table 4 - 9) which indicates that it was within the acceptable range (100 ±10%).

Table 9. The ethanol content of traditional fermented and distilled alcoholic beverage samples.

No.	Beverage type	Location	Ethanol content % (v/v)	Literature report % (v/v)	Reference
1	Yemar Areki	Kotebie	37.5 ± 0.5	-	-
		Kality	38.5 ± 0.5		
2	Gibto Areki	Kotebie	46.0 ± 0.5	46.8 - 50.3	Debebe et al., 2016
		Kality	48.6 ± 0.6		
3	Dagim Areki	Kotebie	51.5 ± 0.5	51.1 - 54.0	Desta, 1977; Debebe et al., 2016
		Kality	51.0 ± 0.5		
4	Wheat Arfa Areki	Kotebie	43.9 ± 0.4	34.0 - 40.0	(Desta, 1977)
		Kality	42.0 ± 0.5		
5	Wheat Sharata Areki	Kotebie	35.9 ± 0.4	34.0 - 40.0	Yohanes et al., 2012
		Kality	37.0 ± 0.3		
6	Dagusa Arfa Areki	Kotebie	48.0 ± 0.5	-	-

		Kality	47.5 ± 0.5		
7	Dagusa Sharata Areki	Kotebie	39.9 ± 0.4	-	-
		Kality	40.9 ± 0.3	8.94 - 13.2	Bahiru et al., 2001; Yohanes etal, 2012
8	Tej	Kotebie	11.9 ± 0.4		
		Kality	13.3 ± 0.3		
9	Shameta	Kotebie	3.23 ± 0.3		
		Kality	3.50 ± 0.5	-	-
10	Bordie	Kotebie	3.00 ± 0.5	-	-
		Kality	2.60 ± 0.4		
11	Wheat Berkraki Areki	Kotebie	42.0 ± 0.6	34.0 - 40.0	Debebe, 2016
		Kality	39.2 ± 0.8		
12	Mixed Areki	Kotebie	40.6 ± 0.6	34.0 - 40.0	Debebe, 2016
		Kality	40.7 ± 1		
13	Koso Areki	Kotebie	41.4± 0.5	42.8 - 56.0	Debebe et al., 2017
		Kality	42.1 ± 0.4		
14	Tella	Kotebie	5.57 ± 0.4	3.50 - 4.50	Yohanes et al., 2012; Berhanu, 2014; Tekluu et al., 2015.
		Kality	6.00 ± 0.5		
15	Korefie	Kotebie	4.43 ± 0.4	2.70 - 2.77	Getnet and Berhanu, 2016.
		Kality	4.50 ± 0.5		

Conditions such as temperature, aeration and strains of the microorganisms obviously affect the level of alcohols. The ethanol content indicates the strength of alcohols. Table 9 shows the variations in the mean concentrations of ethanol of the various samples analyzed. Ethanol concentration of the samples ranged between 2.80% (v/v) - 51.2% (v/v). Among the samples that tested Bordie (traditional fermented beverage) recorded the lowest ethanol content 2.80% (v/v) while Dagim Areki (traditional distilled Areki) showed the highest ethanol content 51.2% (v/v). Ethanol content of factory produced, traditional fermented and traditional distilled beverage samples ranged from 30.0% (v/v) - 41.0% (v/v), 2.80% (v/v) - 12.8% (v/v) and 36.5% (v/v) - 51.2% (v/v), respectively. Ethanol content of Tej were highest compared to others traditional fermented beverages where as Dagim and Ouzo Areki had the highest ethanol content from traditional distilled and factory produced beverages respectively. On the other hand Wheat sharata Areki had lowest ethanol content compared to others traditional distilled beverages where as Bordie and Lomie Areki had lowest ethanol content from factory produced beverages respectively. The mean ethanol content of Vodka, Lomie, Ananas, Ouzo and Dry gin, Yemar, Gibto, Dagim, Wheat Arefa, Wheat Sharata, Dagussa Arefa, Dagussa Sharata, Wheat Berkrakie, Mixed, Koso, Tej, Shameta, Bordie, Tella and Korfie beverages were 39.7, 30.0, 32.0, 41.0, 39.2, 38.0, 47.5, 51.2, 43.0, 36.5, 47.8, 40.5, 40.8, 41.0, 41.7, 12.8, 3.4, 2.8, 5.7 and 5.3% (v/v), respectively. The results show that low content of ethanol were recorded in the traditional fermented beverages as compared to the traditional distilled and factory produced beverages. The oral lethal dose of ethanol had been reported in the range 5 to 8 g/kg. Thus, for a 60 kg adult, 300 g (384 ml) of ethanol can be fatal (Tulashie eta al.; 2017). Therefore, ethanol concentrations of 39.0% (v/v) and above as seen in eleven of the analyzed drinks (Gibto Areki, Dagim Areki, Wheat Arefa Areki, Dagussa Arefa Areki, Dagussa Sharata Areki, Wheat Berkrakie Areki, Mixed Areki, Koso Areki, Vodka Areki, Ouzo Areki And Dry Gin Areki) implies that at least 390 ml of ethanol is contained in 1 L of the alcoholic drink and may all the same cause higher health risks when consumed excessively or without care. The wide variation in ethanol of different beverage type is apparently related to the difference in sources raw materials (crop varieties), limit of standard procedures to produce traditional alcohols, alcohols process conditions and the difference in ferment ability behavior of the beverages (Tulashie et al.; 2017).

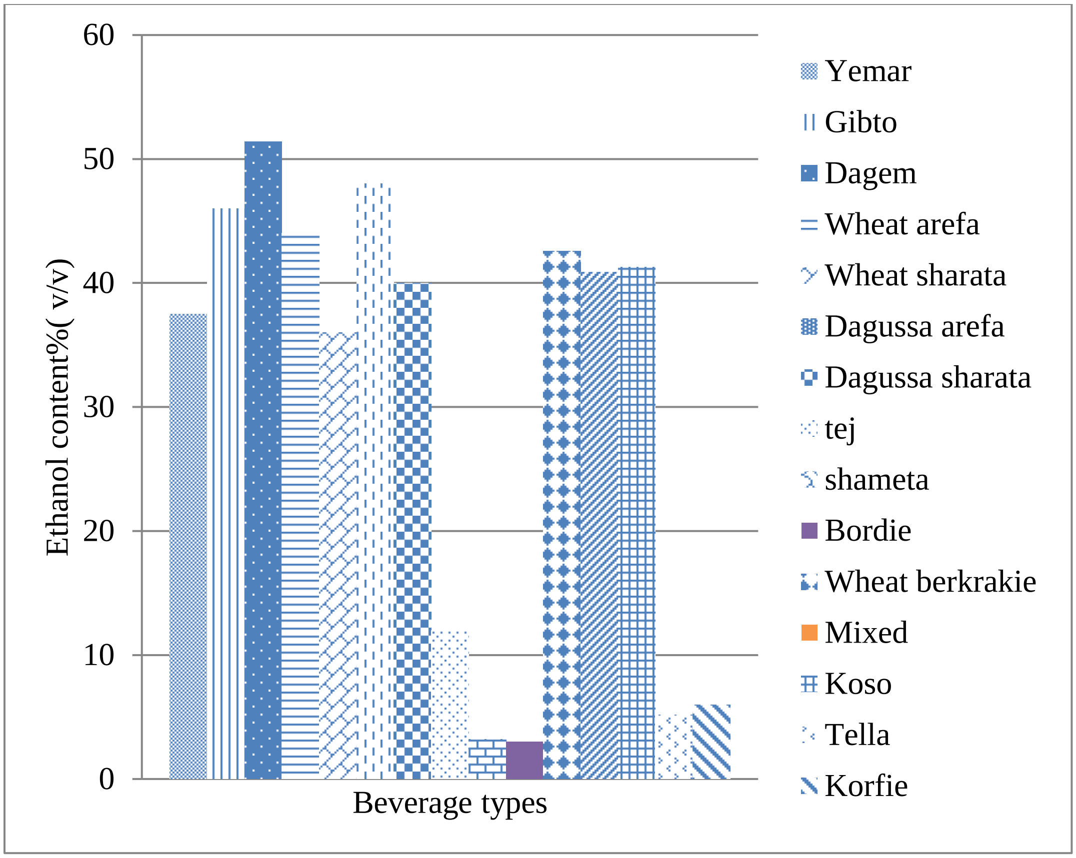

Figure 12. The ethanol content comparison of traditional fermented and distilled alcoholic beverages.

As it can be seen in figure 12, generally the ethanol content across the traditional distilled and traditional fermented beverage types decreased in order of Dagim > Dagussa Arefa > Gibto > Wheat Arefa > Koso > Mixed > Wheat berkrakie > Dagussa Sharata > Yemar >Wheat Sharata> Tej> Tella > Korefie > Shameta > Bordie.

Table 10. Average ethanol content of factory produced alcoholic beverages.

S. No.	Sample type	Amount of ethanol % (v/v)	Labeled amount % (v/v)
1	Vodka	39.7± 0.02	40.0
2	Lomie	30.0 ± 0.01	30.0
3	Ananas	32.0 ±0.09	32.0
4	Ouzo	41.0 ±0.01	40.0
5	Dry gin	39.2 ± 0.03	40.0

Analysis of variance

Analysis of variance (ANOVA) is an extremely powerful statistical technique, which can be used to separate and estimate the different causes of variations. The two-way ANOVA is a means of comparing multiple levels of two independent variables. The two-way ANOVA is grounded in the idea that there are two variables, referred to as factors, affecting the outcome of the dependent variable. Two-way ANOVA statically compares the variations of ethanol content between alcoholic beverages due to the differences in the areas of sample collected or due to their varieties difference. ANOVA uses the F statistic to compare whether the differences between sample means are significant or not. In this study fifteen different types of traditional fermented and distilled alcoholic beverages were collected from two areas of Addis Ababa. During the processes of sample preparation and analysis, a number of random errors may be introduced in each type of alcoholic beverages. The variation in the mean ethanol content of analyze was tested by using ANOVA to see whether the source of the variations was from the varieties of samples or/and due to areas of analyzed sample collected (i.e. differences in topographical location; differences in the mineral contents of the water and atmosphere; variations in the brewing process between women's who prepared alcoholic beverages). The ANOVA results for all the fifteen analyzed alcoholic beverage samples showed that statistically significant differences existed at the 95% confidence level in the mean ethanol content due to the difference varieties of alcohols and significant difference due to the interaction effect of varieties of beverages and areas of sample collection where as areas of alcoholic beverage sample collection was not significant effect at the 95% confidence level in the mean ethanol content for all analyzed alcoholic beverage samples.

Table 11. Analysis of variance (ANOVA) for the ethanol concentrations between traditional fermented and distilled alcoholic beverages.

Source of variations	Df*	SS	MS	F calculated	F critical	Remark
Area	1	0	0	0	4.2	No significance difference in ethanol content between areas of beverages
Varieties	14	24766	2752	19655	4.1	Significant difference in ethanol content between varieties of beverages
Area x Variety	14	22.56	2.51	17.93	4.1	Significant difference in ethanol content between interaction varieties and area
MS within	60	5.47	0.14	-	-	-
Total	89	24793	2754	19673	12.4	-

4.7 Qualitative determination of volatile components of alcoholic beverages

Qualitative data of beverages indicates the type of components present in alcoholic beverages. Twenty different types of beverages were run in GC-MS for qualitative analysis. From the chromatogram many volatile component was identified based on the retention time of components compared with its MS library. In this study methanol is quantitatively determined while other volatile components were qualitatively identified. From the result, ethanol was present in all alcoholic beverages. In Shameta, Tej, Tella, Lomie Areki and Ananas Areki alcoholic beverages, only ethanol was present. In Wheat Berkerakie Areki ethanol, isoamyl alcohol, lactic acid ethyl ester components were present as major components where as methanol, acetic acid, butyl ester, butanoic acid, 1-butanol 3-methyl acetate and furfural were present as minor components. In Vodka Areki ethanol were present as major components where as methanol, isoamyl alcohol, acetic acid butyl ester, butanoic acid were present as minor components. In Wheat Sharata Areki, Dagusa Sharata Areki, Dagim Arekie, Koso Areki, Dagusa Arefa Areki, Yemar Areki and in Mixed Areki ethanol and isoamyl alcohol were present as major components. In Ouzo Areki only methanol and ethanol were present. In Wheat Dagusa Arefa Areki ethanol, isoamyl alcohol, lactic acid ethyl ester and furfural were present as major components. In Gibto Areki ethanol and isopropyl alcohol were present as major components. In Dry gin Areki only methanol and ethanol was present. In Korefie ethanol, Isoamyl alcohol, butanoic acid were present. In Bordie only ethanol was present as major component. Ethanol, Isoamyl alcohol and Lactic acid ethyl ester were the minor volatile components of Bordie.

5. CONCLUSION AND RECOMMENDATIONS

Quantification of methanol concentration in alcoholic drinks showed that some amounts of methanol between the ranges of "not detectable" to 7735 mg/L, however below the minimum oral lethal dose 0.3 – 1.0 g/kg (20 to 60 g or 25 – 75 mL/person in a 60 kg adult). The results indicated that the content of ethanol in beverage samples was ranged from 2.80% (v/v) - 51.2 % (v/v) and there was no significant difference in the ethanol content obtained from the two areas where as there was significant difference in different types of beverages. 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, raw materials used and method of producing (Debebe, 2006). pH value of alcoholic beverage samples ranged from 3.50 - 7.50 but most samples have pH values around 4. Electrical conductivity results of beverage samples ranged from 5.87 - 8391 μS cm^{-1}. Salinity content results of beverage samples were between 0.000 and 4.60%. Total dissolved solid values of tested beverage samples differed between 0.000 mg/L and 4520 mg/L. Total suspended solid results of beverage samples ranged from 0.000 - 16498 mg/L. Total solid values of tested beverage samples differed between 0.000 mg/L and 20328 mg/L. Total acid values of tested beverage samples differed between 0.024 to 105 g tartaric acid/L. The results of volatile acid content (in terms of tartaric acid) of beverage samples ranged from 0.000 to 85.2 g tartaric acid/L. Fixed acid results of beverage samples ranged from 0.018 - 55.9 g tartaric acid/L. Among the twenty types of beverages Shameta, Tej, Bordie, Tella and Korfie had highest acid content, solid content, electrical conductivity and salinity, while it had lowest pH, ethanol and methanol contents than other beverages. Based on qualitative data all fermented beverages had only ethanol whereas distilled beverages had ethanol and other volatile components.

This research work suggests that despite the fact that methanol was found to be present in most of the analyzed alcoholic drinks, the levels observed do not pose any health threat to the human health when consumed, contrary to the general assumptions that it does. However, the normal alcohol health risk associated with high consumption remains a problem. 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 (Ellison et al., 2001). 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 determination of all quality parameters of beverages.

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