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DETERMINATION OF THE LEVEL OF ALCOHOL IN ETHIOPIAN COMMON ALCOHOLIC BEVERAGES USING SPECTROSCOPIC TECHNIQUES

BY

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June, 2013

DETERMINATION OF THE LEVEL OF ALCOHOL IN ETHIOPIAN COMMON ALCOHOLIC BEVERAGES USING SPECTROSCOPIC TECHNIQUES

By

Abel Anberbir

A Thesis Submitted to the
Center for Food Science and Nutrition

In Partial Fulfillment of the Requirements for the
Degree of Master of Science in Food Science and Nutrition

Addis Ababa University
Addis Ababa, Ethiopia
June, 2013

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Approved by the Examining Board **Signature**

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Declaration

This project is my original work except where due reference has been made in the acknowledgments.

Signature

Date July, 10, 2013

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

Name: Dr. Mesfin Redi Signature:

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Date and Place of Submission: Center for Food Science and Nutrition

Addis Ababa University

June, 2013

Abstract

Determination of the Level of Alcohol in Ethiopian Common Alcoholic Beverages Using Spectroscopic Techniques

Abel Anberbir

Addis Ababa University, 2013

A method for the direct determination of ethanol and methanol from Ethiopian distilled alcoholic beverages, *Arekes,* using FT-MIR and UV-NIR in the range 1180–950 cm^{-1} and 1720–1660 nm respectively, were developed. In addition, in the region 3020-2950 cm^{-1} for fermented alcoholic beverages *Tella* and *Tej* ethanol determination were developed and validated. The results obtained were in excellent agreement with the results obtained from Gas Chromatographic measurements. The limit of detection of ethanol and methanol for distilled alcoholic beverages using FT-MIR and UV-NIR methods were 0.025 and 0.007 and 0.019 and 0.038% (*v*/*v*), respectively. For fermented alcoholic beverages, *Tella* and *Tej*, the limit of detection of ethanol was 0.08% (*v*/*v*). The recovery obtained for ethanol using FT-MIR and UV-NIR ranges from 99-102 and 98-105% (*v*/*v*), respectively, for distilled alcoholic beverages and 99 – 103 and 101 - 102% (*v*/*v*) for *Tella* and *Tej*, respectively. The average ethanol level found in traditional Ethiopian alcoholic beverages, *Arekes*, *Tella* and *Tej* were 31.6 – 51.4, 1.94 -5.65 and 6.79 - 9.36% (*v*/*v*) respectively. In all samples methanol was not detected.

Key words: Ethanol; Methanol; *Areke; Tella, Tej*; FT-MIR and UV-NIR Spectrophotometers

Acknowledgements

It gives me great pleasure to express my deepest gratitude to my advisors Dr. Mesfin Redi and Prof. Nigussie Retta for their unreserved guidance, and encouragement. I am also grateful to the Center for Food Science and Nutrition and the Department of Chemistry (Addis Ababa University) for providing with me all the facilities available to them

I am also indebted to Ayalew Debebe, Kehali kirkos, and all food science and nutrition staff memebers for their immense help in all laboratory work.

My special thanks go to the Ethiopian Conformity Assessment Enterprise (ECAE) Management teams (especially for Mr. Gashaw Tesfaye) for giving me the opportunity to join the school of graduate studies. I am also grateful to ECAE Biochemical testing laboratory members.

My deepest gratitude and love goes to all my friends, especially to Abdi Duga, Abebe Getu, Ashenafi Ambaw, Yohannes Seyoum, Belete Eshetu, Dawit Tibebu, Engedasew Temere, Feaza Seid, Adil Ibrahim and Temnit Amanuel for being great friends and for the wonderful moments we had.

My heartfelt thanks also go to my sisters (Rita and Sara) and brother (Yohannes). At last, but by no means least, my genuine thanks and love go to my parents (Anberbir Shibeshi and Genet Tesfaye), for their love, care, and help that enabled me to realize my long aspired dream.

Dedicated to my parents

Anberbir Shibeshi and Genet Tesfaye

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List of Abbreviations and Acronyms

EBC	European brewery convention
FT-MIR	Fourier Transform-Midinfrared
GC-FID	Gas chromatography with Flame Ionization Detector
LOD	Limit of detection
LOQ	Limit of quantification
NATA	National Association of Testing Authorities
r^2	Coefficient of determinations
SD	Standard deviation
UV-NIR	Ultraviolet-Near Infrared
WHO	World Health Organization

1. Introduction

1.1 Background

Alcoholic beverages have been widely consumed since prehistoric times by people around the world, as a component of the standard diet, for hygienic or medicinal reasons, recreational purposes, and for other reasons. In nearly all parts of the world, some type of alcoholic beverage native to its region is prepared and consumed. In Africa, fermented alcoholic beverages are consumed in different occasions such as marriage, naming and rain making ceremonies (Zvauya, Mygochi, & Parawira, 1997).

Alcoholic beverages, fermented and distillated liquors are complex mixtures mainly consisting of ethanol up to 60% (*v/v*) (Kuria & Olando, 2012; Tipparat, Lapanantnoppakhun, Jakmunee, & Grudpan, 2001; Wang, Choong, Su, & Lee, 2003) water and a large number of minor compounds such as alcohols, acids, esters, aldehydes, polyphenols, metals, amino acids, etc (Madrera & Valles, 2007). Distilled alcoholic beverages are characterised by the presence of volatile compounds (fusel alcohols, fatty acids, esters and others), that affect sensorial properties such as odor, taste and color (Dragone, Mussatto, Oliveira, & Teixeira, 2009; Savchuk et al., 2001).

Alcoholic beverages apart from serving as inebriating drinks are consumed in different occasions (Bahiru, Mehari, & Ashenafi, 2001; Lyumugabe, Bajyana Songa, Wathelet, & Thonart, 2013; Zvauya et al., 1997). Consequently, they have positive and negative effects. The harmful use of alcohol results in approximately 2.5 million deaths each year, with a net loss of life of 2.25 million, taking into account the estimated beneficial impact of low levels of alcohol use on some diseases in some population groups (Dept, 2004)

Harmful drinking can also be very costly to communities and societies. Alcohol consumption is the world's third largest risk factor for disease and disability; in middle-income countries, it is the greatest risk. Alcohol is a causal factor in 60 types of diseases and injuries and a component cause in 200 others (Dept, 2004). For instance, methanol is highly toxic volatile (Pérez-Ponce & de La Guardia, 1998) cause headaches to blindness and even death from an ingestion of 100-250 mL (Cabaroglu, 2005; Garrigues, Pérez-Ponce, Garrigues, & De la Guardia, 1997; Pérez-Ponce & de La Guardia, 1998). The positive effects are associated with moderate consumption and with low ethanol content of certain alcoholic beverages, mainly wine and beer (Prompona, Kandylis, Tsakiris, Kanellaki, & Kourkoutas, 2012).

Indigenous African fermented alcoholic beverages include Egyptian *bouza*, Tanzanian *Wanzuki, gongo, tembo-mnazi* and *gara*, Nigerian *palm-wine*, Kenyan *muratina* and *uragua*, and South African kaffir beer (Bahiru et al., 2001).

Indigenous Ethiopian traditional alcoholic beverages *Tella, Tej, korefe, Areke*, etc. vary in their ingredients and level of alcohol. *Tella* (Ethiopian home-brewed beer) is brewed with barley or wheat, hops and its alcoholic content ranges from 2 to 8% (*v/v*) (Sahle & Gashe, 1991). *Tej* (indigenous Ethiopian honey wine) is a home-processed, honey wine. It is prepared from honey, water and leaves of gesho. The alcoholic content of *Tej* is between 6.98% and 10.9% (v/v) (Bahiru et al., 2001). *Areke* is a distilled beverage made from gesho and different grains (cereal based only) and some other flavouring ingredients like garlic, kosso (*Hagenia Abyssinica*), honey, etc., (Fite & Tadesse; Sahle & Gashe, 1991).

The use of the local traditional brews poses a danger to the society for a number of reasons. Firstly, the manufacture process of the traditional brews is usually unhygienic and at times the brews are laced with methanol resulting deaths, blindness and disabilities. According to (R. N.Simiyu & 2010) in Kenya, Methylated brews, made by mixing non-beverage alcohols such as methanol with other ingredients for example, chang'aa (distilled sprit), untreated water, caramel, colourings, sugar and flavourings are consumed. The concoctions are sold cheaply with disastrous consequences.

Ethiopian traditional alcoholic beverages are produced mostly in small scale vending houses for different occasions. Because these beverages are largely outside government control, there is no information on their production quality and the levels of alcohols and therefore, the consumer are at risk of different health problems. Alcohol consumption is highest in poor communities where potent home brewed alcohol is cheap and readily available (Parry, Patra, & Rehm, 2011). There is an overwhelming proliferation of uncontrolled alcoholic brews both in the urban and rural parts of Ethiopia including: Debrebrhan, Butagera, Arsi- Negele, Dembecha and the capital of Ethiopia.

Numerous analytical methods are already available for determination of alcohol levels in alcoholic beverages. These techniques vary greatly in their preparation, accuracy, precision, use, cost, environmental friendliness and overall practicality (Wang et al., 2003).

The widely employed techniques for determining the level of alcohol in beverages include densitometric (AOAC, 2009a; Wang et al., 2003), refractive index (Wang et al., 2003), oxidation of distillate (Wang et al., 2003), dichromate oxidation (Wang et al., 2003), enzymatic (Wang et al., 2003), biosensor (Rotariu, Bala, & Magearu, 2004; Wang et al., 2003), potentiometry (OHURA, IMATO, IsHIBASHI, AsANO, & YAMASAKI, 1990; Wang et al., 2003), gas chromatography (AOAC, 2009c; Wang et al., 2003), capillary electrophoresis (Wang et al., 2003), high performance liquid chromatography (Kuo, Wen, Huang, Wu, & Wu, 2002; Wang et al., 2003) beer analyser (Wang et al., 2003), colorimetric method (Sumbhate, Nayak, Goupale, Tiwari, & Jadon, 2012) and flow injection analysis (Wang et al., 2003). However, the techniques employed are tedious, expensive, time consuming and require sample dilution (Tipparat et al., 2001).

Infrared spectroscopy based methods are recently emerging because of their versatility, efficiency, low cost, fast and noninvasiveness (Nagarajan, Gupta, Mehrotra, & Bajaj, 2006). Hence, IR spectrometers have been used for direct determination of methanol and ethanol in alcoholic beverages. But, in gaseous (Garrigues et al., 1997)which requires costly apparatus, additional and carrier gas and thus the analysis is costly.

The objective of this study, is therefore, the determination of the level of alcohol in Ethiopian common alcoholic beverages (*Areke, Tella* and *Tej*) using optical spectroscopic techniques.

1.2 Statement of the problem

According to disaster management and risk reduction (2010), methylated brews, made by mixing non-beverage alcohols such as methanol with other ingredients for example, chang'aa of Kenya (distilled sprit), untreated water, caramel, colourings, sugar and flavourings. The concoctions are sold cheaply with disastrous consequences. Methylated brews are common in rural area and slum areas. Over 140 Kenyans in Nairobi in the neighborhoods of Mukuru Kwa Njenga and Mukuru Kayaba slums were died in 2000 after consuming a brew called kumi kumi or “ten ten”.

In Ethiopia the consumption of Common alcoholic beverage such as Tej, Areke, Tella is increasing day to day without determining the level of alcohol and without labeling. In addition almost all the traditional alcoholic beverage vending houses are out of government control. Since, Ethiopian traditional alcoholic beverages are produced mostly in small scale vending houses for different occasions. Accordingly, there is no information on their production quality and the levels of alcohols are not known. Due to this the consumer are at risk of different health problems.

According to European Commission Directive 87/250/EEC the alcoholic strength indication using a symbol '% vol.' became mandatory in the labeling of alcoholic beverages (Commission, 1987). For this reason, determination of the density has been used for a long period of time as the approved reference method to determine the alcoholic strength in different alcoholic beverages. Since, the densitometric measurement typically has to be preceded by a distillation step which is time-consuming and also require special training of personnel to obtain a valid and reproducible result, because there is a risk to cause experimental errors during distillation steps and subsequent densimetric measurements.

During the last two decades, most of the researches on alcoholic content determination were focused on spectroscopy techniques. Recently, fast and multi-component analysis using fourier transform infrared spectroscopy (FTIR) have been reported (Lachenmeier, 2007).

According to European brewery convention (EBC) and Ethiopian standard, the distillation method is regarded as the official reference method (Agu, 1995). The following disadvantages speak against the distillation method as a routine analytic method: first, it is a time-consuming method; second, it has a restricted degree of automation; and, third, a degree of operational variation is incorporated in the system.

Therefore, to combat the above problems it is important to develop and validate spectroscopic techniques (UV-NIR and FT-MIR) for the determination of Ethiopian common alcoholic beverages alcohol levels.

1.3 Significance of the study

Today, traditional beverages industry is a huge business, consisting of several private small scale industries and many thousands of vending producers ranging from Tella to Areke. The Common alcoholic beverage industry such as Tej, Areke, Tella in Ethiopia is increasing day to day without determining the level of alcohol and without labeling the strength of alcohol. In addition there is no regulations and standard test method for analysis of the alcohol levels for Ethiopian traditional alcoholic beverages.

FT-MIR and UV- NIR spectroscopy methods for the direct analysis of ethanol and methanol in Ethiopian common alcoholic beverages having reliably, fast and accurate results were developed and validated in this study. Therefore the developed and validated analytical methods of FT-MIR and UV-NIR spectroscopic techniques fill the gap of very old and time consuming pycnometric and densitometer test methods.

1.4 Objectives

1.4.1 General objectives

The main objective of the present study is to determine the level of ethanol and methanol in common Ethiopian alcoholic beverages, *Areke, Tella* and *Tej*, using liquid phase FT-MIR and UV-NIR spectrophotometery and to assess the health impacts of uncontrolled consumption of alcoholic beverages.

1.4.2 Specific objectives

1. To develop fast and low cost analytical methods for the direct determination of ethanol and methanol in common Ethiopian alcoholic beverages (*Areke, Tej and Tella*) by using liquid phase FT-MIR and UV-MIR spectrophotometery.
2. To validate the developed methods
3. To quantify the alcohol contents in Ethiopian alcoholic beverages using the newly developed and validated methods based on FT-MIR and UV-NIR spectrophotometric techniques
4. To assess the health impact of uncontrolled consumption of alcoholic beverages.

2. Literature review

Alcoholic beverage is defined as "the product known as ethyl alcohol obtained by fermentation and distillation of any fermented alcoholic product". "Alcoholic drink includes spirits, wine, beer, traditional alcoholic drink and any one or more such varieties containing one-half of one percent or more of alcohol by volume". Worldwide there are three common types of alcoholic beverages namely, beer, wine and spirits. Beer and wine have lower alcohol content as compared to spirits (Kuria & Olando, 2012).

Ideally the definition of alcohol should be at an ethanol content level low enough to include most of the alcoholic beverages consumed in any country. Such definition takes into consideration the alcohol by volume content of the drink Amounts of alcohol consumed by a person depend partly on the ethanol content. Estimates of both mean volume of alcohol consumption and heavy drinking amounts are influenced by variation in alcohol concentration and quantity. People from poor com- munities prefer cheap and potent alcohol. The use and abuse of alcohol in Africa involves both local and industrialized types of alcohol with as much as half of the consumption being unrecorded alcohol.

Traditional fermented beverages are those that are indigenous to a particular area and have been developed by the local people using an age-old techniques and locally available raw materials (Bacha, Mehari, & Ashenafi, 2004).

Indigenous fermented alcoholic beverages from different parts of the world are described by Steinkraus, (1983) and some of the indigenous African fermented alcoholic beverages include Egyptian *Bouza*, Tanzanian *Wanzuki, Gongo, Tembo-mnazi* and *Gara*, Nigerian

Palm-wine, Kenyan *Muratina* and *Uragua*, and South African *Kaffir* beer (Bahiru et al., 2001).

Ethiopia is one of the countries where a variety of traditional fermented foods and beverages are produced and consumed. The beverages are produced in home usually for local consumptions. Among Ethiopian fermented beverages are varieties of *Tella, Tej, Areke, Korefe, Borde, Shamita* and *Keribo* (Bahiru et al., 2001).

Of traditionally fermented beverages in Ethiopia, the most popular alcoholic beverages are *Tej*, *Tella* and *Areke.* These drinks are widely served on festive occasions and at social gatherings. Traditional recipes are handed down through generation and are still used for food processing in many developing countries (Abegaz, Beyene, Langsrud, & Narvhus, 2002).The traditionally fermented beverages are low-cost product in all aspect as they are usually manufactured using only rudimentary equipment. Because of their cheapness, low-income groups mostly consume them. Thus their handling and consumption often takes place under conditions of poor hygiene (Steinkraus, 1983).

2.1 Common Ethiopian traditional beverages

2.1.1 *Tella*

Tella is an Ethiopian home-brewed beer prepared from different ingredients and which differs from the others in some respects. It is the most commonly consumed alcoholic beverage in Ethiopia (Gizaw, 2006). First it is brewed with barley or wheat, Gesho (*Rhamnus prenoids*). Secondly, it has a smoky flavour 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 2 to 4% (*v*/*v*).

Tella is a beverage of variable viscosity and having a variety of colors (ranging from grayish-white to dark brown). According to Gizaw research in 2006, the mean alcohol content of *Tella was* 6.36% (*v*/*v*). The mean values for methanol, fusel oil, and ethanol were found to be 35 ppm, 66 ppm, and 3.6%, respectively.

Filter *tella* is another beverage, which is made in the same way as the regular *tella*, but it is more concentrated. The *Tella* is then filtered through a cotton cloth. After being prepared it is kept in a closed container. This type of *Tella* is reported to have higher alcohol content. According to Desta (1977) the average alcohol content of filter *Tella* is 11.47% (*v*/*v*), with the range of 8.91 – 14.52% (*v*/*v*). *Kirari* is a drink made, when the clear *Tella* is used, by adding fresh water and then leaving the mixture to ferment. This beverage is weaker than the regular *Tella*, and is most often used for family consumption (Desta, 1977).

2.1.2 *Tej*

Tej (indigenous honey wine) is a home-processed, but also commercially manufactured. It is prepared from honey, water and leaves of Gesho (*Rhamnus prenoids*). 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 colour similar to that made from honey (Bahiru et al., 2001).

Tella (homebrew beer) and *Tej* is the typical Ethiopian honey-wine or mead. *Tej* are known under various names, differing according to language. Tej or daadhii (in Oromo) has become the most popular drink of many Ethiopians, not only in towns but also in countryside bars. In popular bars it is, and often still is, served in longneck glass bottle, (*Berelle)*, old perfume bottles and a custom dating from the late 19th century. The best *Tej* is considered to be the filtered kind. Yellow honey is usually preferred for *Tej* and *Berz*, a non-fermented honey solution. Although economically not so significant, tej is informally exported through country visitors and transitory (Kloman, 2010).

Fermentation of *Tej*, like other traditionally fermented alcoholic beverages, relies on the microorganisms present in the substrates and equipment. As these fermentations are natural and, thus, uncontrolled, alcohol and fusel oils produced during the fermentation can be hazardous to health if produced beyond acceptable levels. With the variable microflora of such spontaneous fermentation, variability of the product is inevitable (Bahiru et al., 2001).

According to (Gizaw, 2006) the average pH and ethanol content of *tej* is 3.77 and 11.47 respectively and the mean value of methanol and fusel oil, for *tej* samples collected from different regions of Ethiopia were 47 ppm and 104 ppm, respectively. According to Bahiru et al., (2001), the fusel oil content of *'tej'* samples varies between 0.1 g/l00L and 88 g/100L.

2.1.3 *Areke*

Areke is a distilled alcoholic beverage. It is a colorless, clear, traditional alcoholic beverage which is distilled from fermentation products prepared in almost the same way as *Tella* except that the fermentation mass in this case is more concentrated (Fite & Tadesse).

Areke 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 *Areke* are predominantly lower class people or those who have become dependent upon alcohol and cannot afford to buy industrially produced alcohol (Dept, 2004). Traditionally *Areke* is classified into two: *Terra-Areke* and *Dagim-Areke.* 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' (Gizaw, 2006).

Terra-Areke (ordinary- Areke) is a colorless, clear, local alcoholic beverage, which is distilled from a fermentation product of different grains and hops known as *Tensis* (Desta, 1977). *Areke -tensis* is prepared by mixing powdered *Gesho* leaves *(Rhamnus prenoids)* and powdered wheat malt *(Bikil)* with water to give a mixiture of free flowing consistency, and which will be put aside to ferment for about five days. An amount of *Dagussa* (*Elusine coracann*) roughly equivalent to four times that of the *Bikil*, is powdered kneaded with water to make dough and baked into cakes. The hot cakes are broken into pieces, added to the first mixture and with more water, well mixed and again left aside to ferment for about four days. Portions of the second mixture are transferred to the traditional distillation apparatus and distilled to give what is known as *Terra-Areke.* According to Gizaw (2006), *Terra-Areke* has mean alcoholic content of 37.22% (*v/v*), with a range of 30.20 - 39.80% (*v/v*).

Dagim-Areke is a stronger type of *Terra-Areke*, which is prepared in the same way as *Terra-Areke*, except that the distillation process is allowed to proceed for a shorter period of time, or three volumes of *Terra-Areke* are redistilled to give about one volume of *Dagim-Areke*. The redistilled *Areke* will then have higher alcohol content. The average alcohol content of *Dagim Areke* is around 48% (*v/v*) (Gizaw, 2006).

Since the government has no control over the production of locally brewed alcoholic drinks, it is difficult to estimate the amount of alcohol production and consumption in Ethiopia (Selinus, 1971). However, the unrecorded alcohol consumption is estimated to be 1.0-liter pure alcohol per capita for population older than 15 years of age for the years after 1995 (Dept, 2004).

According to Gizaw, (2006) methanol content in the samples of Dagim *Areke, Tella, and Terra Areke* were not detected. In the studies conducted so far on the local beverages, however, the methanol content was reported in the ppm level; *Areke* (320.87 ppm), *Tella* (32.37 ppm), *Tej* (45.67 ppm) (Fite & Tadesse) .

2.2 Uncontrolled alcohol consumption and its effect

Unrecorded alcohol refers to alcohol that is not taxed and is outside the usual system of governmental control, because it is produced, distributed and sold outside formal channels. Unrecorded alcohol consumption in a country includes consumption of homemade or informally produced alcohol (legal or illegal), smuggled alcohol, alcohol intended for industrial or medical uses, alcohol obtained through cross-border shopping (which is recorded in a different jurisdiction), as well as consumption of alcohol by tourists (Rehm, Kanteres, & Lachenmeier, 2010).

The consumption of homemade or illegally produced alcohol may be associated with an increased risk of harm because of unknown and potentially dangerous impurities or contaminants in these beverages. Homemade or informally produced alcoholic beverages are mostly fermented beverages made from sorghum, millet, maize, rice, wheat or fruits. According to World Health Organization the average consumption of unrecorded alcohol in Ethiopia per capita of adults between 2003 and 2005 was 3.5 liters of pure alcohol (Alwan, 2011).

Non standardized brews affect the health status in different parts of the world. Since, some respondents were aware that some of the brews were too strong and even 'poisonous' for health, especially chang'aa (*Chang'aa* is an illegal alcoholic drink which is distilled from grains like maize and sorghum and sometimes adulterated with jet fuel, battery acid to accelerate fermentation and make it more potent). It may cause death or blindness when drunk. Preparation of busaa on the other hand involved use of water, whose source was not always safe (Authority, 2010).

Several studies in developing countries have revealed such additives; for example, methanol found in illicit brews in Brazil (Harworth, 2004) methanol and valium in Pakistan; aftershaves and fire lighting fuels in Estonia and a very high alcoholic content of up to 60.5% in India (Botha, 2009).

Different types of additives in Kenya was added which includes sisal juice, battery water, disinfectants and others (R. N.Simiyu & 2010).

Alcoholic beverages vary considerably in ethyl alcohol content and their primary ingredients. The establishment of the analyte in social interactions caused regulations of alcohol concentration in beverages due palatability and health factors.

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

It has often been observed that alcoholism is a more significant problem than all other forms of drug abuse combined (Desta, 1977). According to some studies carried out in some African countries there is considerable evidence that home produced alcohol drinks are known to have toxic components (Fite & Tadesse).A report from Zambia 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 and fusel oil were shown to be the common contaminants of traditional alcoholic beverages in the studies carried out so far. Methanol is highly toxic and can cause blindness, insanity and even death, depending on the amount consumed. Toxic effects are usually associated with a methanol concentration in blood greater than 100g/mL (Reilly, 1974).

Fusel oil is a collective name of isopentyl alcohol, 2-methyl-1-butanol, isobutyl alcohol, propyl alcohol, esters and aldehydes. It is toxic and has been shown to cause cancer in experimental animals. These alcohols are responsible for the severe headache and thirst associated with hangover and also account for taste and flavor of alcoholic drinks (Fite & Tadesse).

In Ethiopia there are different type of traditionally fermented beverages, the most popular alcoholic beverages are *Tej* (honey wine), *Tella* (a malt beverage like beer) and *Areke*. These drinks are widely served on festive occasions and at social gatherings (Bahiru, Mehari et al. 2001).

The traditionally fermented beverages are low-cost product in all aspect as they are usually manufactured using only rudimentary equipment. Because of their cheapness, low-income groups mostly consume them. Thus their handling and consumption often takes place under conditions of poor hygiene (Steinkraus, 1983).

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2.3 Regulations on the composition of alcoholic beverages

In general, the standards provide some information about suitable additives for alcoholic beverages with maximum levels for certain substances. Maximum levels are also given for certain biologically active substances in natural flavorings. Due to advances in food production and surveillance, the concentrations of some contaminants (e.g. nitrosamines in beer, lead in wine) have been significantly reduced over the past years. The standards have been incorporated into the national legislation of the majority of countries including Ethiopia. However, some countries may impose more specific or more stringent regulations (Lachenmeier, 2007).

2.4 Methods of analysis of Alcoholic beverages

Quality standardization is an essential task in the liquor production industry. There have been several techniques used for standardization based on the various physical and chemical properties of alcoholic beverages.

The alcoholic strength expressed in percent by % (v/v) is one of the oldest parameters for which quantitative analytical methods have been developed, and is still determined in businesses and laboratories around the world. The densimetric measurement typically has to be preceded by a distillation step (especially for beer, wine and liqueurs), because sugars and other solutes would otherwise lead to false results, as the tables for converting density to alcoholic strength are based on pure water-alcohol mixtures. Rapid and mobile determination of alcoholic strength in wine, beer and spirits using a flow-through infrared sensor was developed (Lachenmeier et al., 2010).

During the last 20 years, most of the research on alcoholic strength determination was focused on spectroscopy. The earliest infrared (IR) spectroscopic methods typically applied Fourier transform infrared (FTIR) or near infrared (NIR) spectrometers and simple linear or multi-linear models to derive the alcoholic strength from the ethanol bands, typically of the first or second-order derivative (Lachenmeier et al., 2010).

Fourier Transform Infrared (FTIR) spectroscopy in combination with multivariate data analysis is also introduced for the quality control and authenticity assessment of spirit drinks and beer in official food control. The spectra were measured using a FTIR interferometer, which is purpose-built for the analysis of alcoholic beverages and includes an injection unit for liquids with automatic thermostating of the sample. Only 2 min are required for FTIR measurement. For spirit drinks, no sample preparation is required at all (Lachenmeier, 2007).

Compared to densimetric reference methods, infrared sensors are much simpler to handle, and also appear to be suitable for industrial process control. The possibility for mobile use offers opportunities in changing locations or even on-site inspections. The device can be successfully applied to labelling control of wine, beer, and spirits, for the monitoring of fermentations, and for the evaluation of unrecorded alcohols (Lachenmeier et al., 2010).

Fourier transform (FT)-near infrared and FT-Raman spectrometry by the partial least squares (PLS) calibration models for the determination of the ethanol content of ethanol fuel and alcoholic beverages. The result shows of FT-NIR model has an accuracy equivalent to that of the reference method in the analysis of ethanol fuel, while in the analysis of beverages, the FT-Raman model presents an accuracy equivalent to the reference method. The limits of detection for NIR and Raman calibration models were 0.05 and 0.2% (*w/w*), respectively. According to the researcher the result shows that both techniques, present better results than gas chromatography (GC) in evaluating the ethanol content of beverages (Mendes, Oliveira, Suarez, & Rubim, 2003) .

Simultaneous vapour-phase FTIR determination of methanol and ethanol were studied based on the use of proportional equations, obtaining quantitative data with limits of detection of 0.21% (v/v) for ethanol and 0.04% (v/v) for methanol. The recovery of methanol in natural samples, extremely scattered values were reported for low methanol concentrations between 0.22 and 0.45% (*v/v*) in the presence of ethanol levels higher than 37% (*v/v*), the average values of 100 ± 50, 106 ± 40 and 102 ± 30% (*v/v*) and additionally in some cases extremely low recoveries such as 59 ± 50% or values as high as 118 ± 13% (*v/v*) were reported (Pérez-Ponce & de La Guardia, 1998).

According to Garrigues in (1997), FTIR procedure is proposed for the direct determination of methanol and ethanol in liquid samples of alcoholic beverages, based on vapor generation from small injected volumes of untreated samples into a heated Pyrex glass reactor in which, at a temperature of 80°C, ethanol and methanol are volatilized and introduced into a long-path IR gas cell by means of a N_2 carrier flow. The IR spectra obtained present two characteristic ethanol bands (1050 and 880 cm^{-1}) and a single characteristic methanol band (1030 cm^{-1}). The measurement of the area of the transient recording obtained for the wavenumber ranges between1025 – 950 and 950 – 820 cm^{-1} allows us the determination of ethanol and methanol, respectively in the same sample by using a simple proportional equations approach. However the proposed method is affected by temperature, carrier gas flow rate and injection volume and also the technique requires carrier gas, N_2, which makes the method expensive. In addition the limit of detection for ethanol and methanol were 0.21 and 0.04% (*v/v*), respectively (Garrigues et al., 1997).

Another researchers Tipparat in (2001) also developed near-infrared (NIR) spectrophotometer with flow injection (FI) method was as a simple procedure for the determination of ethanol in a liquor. A liquor sample is equilibrated off-line with dried chloroform to extract ethanol into the organic phase. The extract is injected into a carrier stream of dried chloroform passing through a home-made flow through cell (1 mm path length) sitting in a NIR spectrophotometer for continuous monitoring of absorbance at 2305 or 2636 nm. The ethanol content can be evaluated from a calibration established by a plot of change in absorbance versus concentration of ethanol standard solutions. A calibration is linear in the range of 20 – 50% (*v/v*) ethanol. However, the method requires extraction with chloroform with poor limit of detections (Tipparat et al., 2001). According to Gallignani (1994) research FTIR spectrometry provides a direct method for the fast and accurate determination of ethanol in beer samples and does not require any chemical pre-treatment of the sample. The use of the uncorrected second-derivative values provides accurate and reproducible results in the analysis of regular beer samples with an ethanol concentration between and 7.5% (*v/v*). However, for the analysis of low-alcohol beers, the use of the first-derivative data provides better reproducibility. In this instance, the experimental measurements for ethanol, obtained using the first derivative, must be corrected, taking into account the maltose concentration and the relationship between maltose and ethanol (Gallignani, Garrigues, & de La Guardia, 1994).

A simple and rapid method was developed to determine ethanol content in alcoholic beverages using megapore polar column (CPWax 58 CB, 30 m x 0.53 mm) with direct injection gas chromatography. Ethanol in sample was injected directly into GC and required sample preparation. The proposed method has higher precisions, accuracy and sensitivity. However, the methods requires internal standards, and sample preparation (Wang et al., 2003) .

Analytical method used to characterize the quality of distilled drinks should to perform an analysis without sample pre-treatment. In addition, it should to accomplish a fast data acquisition and carry out the data treatment accurately with relatively low costs. The application of spectroscopic methods is a good way to reach these premises (Barboza & Poppi, 2003).

2.4.1 FT-IR and NIR spectrophotometers

A Fourier transform is a mathematical operation used to translate a complex curve into its component curves. The complex curve is an interferogram generated by overlapping light waves, the standard infrared spectrum is calculated from the Fourier-transformed interferogram, giving a spectrum in percent transmittance (%T) versus light frequency (cm^{-1}).

Fourier techniques basically differ from conventional techniques in that they measure radiant power as a function of time (time domain) whereas conventional spectroscopy measures power as a function of frequency (frequency domain). This time domain spectrum is then mathematically converted into a frequency domain spectrum using a Fourier transform. The process is so complex that it requires a high speed computer and will not be covered here (Stuart, 2004).

There are three major advantages of using Fourier Transform techniques in IR spectroscopy (Lachenmeier, Sarsh, & Rehm, 2009; Stuart, 2004)

- Fourier transform instruments do not need slits to attenuate radiation and have fewer optical elements. The increased power reaching the detector gives a larger signal to noise ratio
- The high resolving power and wavelength reproducibility allow for more accurate analysis of collected spectra
- The multiplex advantage (or faster scanning). In Fourier techniques, all wavelengths are scanned simultaneously, allowing an entire spectrum to be scanned in 1 second or less. Since the signal to noise ratio increases as the number of scans, *N*, increases, thus Fourier techniques allow many more scans in less time and much better signal to noise ratios.

Similarly, NIR spectroscopy gives much information for more than 30 components including ethanol, water, organic acids, higher alcohols, etc. in alcoholic beverages. With no prior preparation of the samples is required for distilled alcoholic beverages, spirits (Lachenmeier, 2007).

Near-infrared (NIR) spectroscopy is characterized by low sensitivity, thus, its application range is limited to the principal constituents. Pure alcoholic beverages (whiskey, brandy, rum and vodka) and those adulterated with 5 % and 10 % (v/v) of water, ethanol or methanol were successfully classified using NIR spectroscopy and chemometric methods (Barboza & Poppi, 2003).

The reference procedure for determining alcoholic strength by volume consists of a distillation followed by a density measurement using pycnometry, electronic densimetry. This requires relatively large liquid volumes of up to 200 ml, which are often not easily available in samplings of unrecorded alcohol, and also depletes samples volume unnecessarily (Lachenmeier et al., 2010).

The application of spectroscopic techniques in the study of the origin and differentiation of food and drink products has developed considerably in recent years. The advantage of these techniques lies in the almost complete lack of sample preparation required, which makes them especially rapid to apply (Palma & Barroso, 2002). Thus, the alcoholic strength is to be determined by the most efficient Fourier transform infrared (FTIR) spectroscopy, which is faster and uses a lower sample amount, with necessary precision and accuracy (Lachenmeier et al., 2010).

Therefore, the main objective of this work was developing a new method for the direct determination of ethanol and methanol in distilled and fermented Ethiopian alcoholic beverages with simple, accurate, economic and rapid techniques of UV-NIR and liquid phase-FT-MIR without sample preparation.

3. Materials and Methods

3.1 Locations of the Study

In this study samples of *Areke* were collected from where *Areke* is widely produced and consumed areas including Addis Ababa, Arsi Negele, Butajira, Debrebrhan, and Dembecha. The commercial samples were supplied by National alcoholic beverages industry. The fermented alcoholic beverages *Tella* and *Tej* were collected from different parts of Addis Ababa. The study areas are indicated in Figure1.

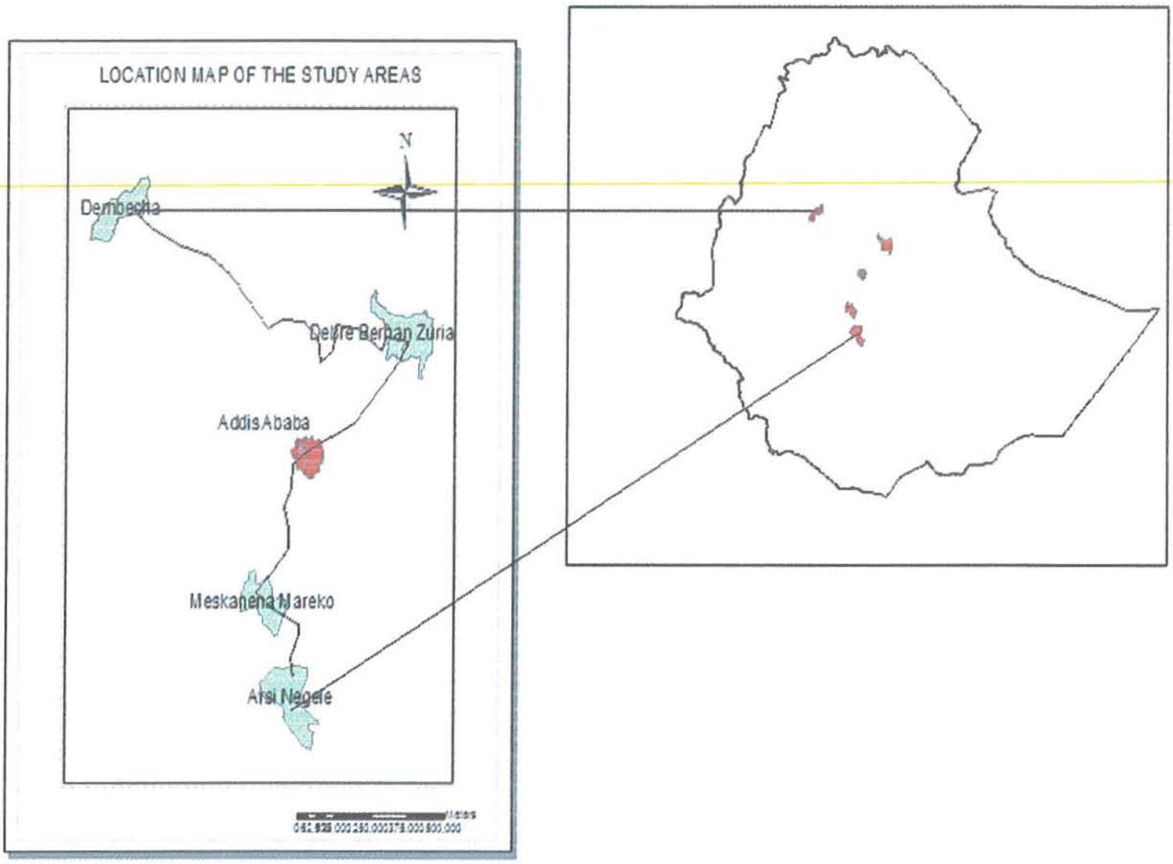


Figure 1. Map of the areas where the sample were collection: Addis Ababa, Arsi-Negele, Butajira, Debreberhan and Dembecha

3.2 Apparatus and Reagents

UV-Vis/NIR spectrophotometer (Lambda 950, Perkin Elmer, UK), Fourier Transform infrared spectrometer (Spectra 65, Perkin Elmer, UK) and Gas Chromatography (GC 1000, Dani, Italy) were used for the determination of ethanol and methanol contents. Density meter (DMA 4500M, Anton Paar, Austria) was used to measure the density of methanol and ethanol standard solutions. ZnSe windows and Quartz cuvette of 10 mm optical pathlength were used as sample holders for FTIR-MIR and UV-NIR, respectively. The pH of the samples were measured using pH meter (MP511 Lab pH meter, China), and analytical Balance (Adventurer, OHAUS, China) was used for weighing purposes.

Methanol (99.7%, Sigma-Aldrich, France) and Ethanol (99.99 %, Fisher Scientific, UK) were utilized to prepare standard solutions. D (+) - Glucose (99.95%, Merck, Germany), D-Fructose (99.95% Pharamacos Ltd., England), Sucrose (99.9%Pharamacos Ltd., England), Potassium hydrogen phthalate (99.99% Merck, Germany) and distilled water (from the chemistry department Addis Ababa University) were used for dilution of samples.

3.3 Sampling and sample preparations

Traditional and commercial samples were analyzed. *Areke* samples were collected from varies parts of Ethiopia where they are produced and widely consumed. The collected samples of *Areke* include: Yekoso (*Hagenia Abyssinica*), Yemar (Honey Areke), Cereal (only cereal based), Dagim (double distilled), Yetenaadam and Nechshinkurt (*Ruta Chalepensis*) and (*Allium Satiuum* or garlic) respectively, and Yegibto (*Lupinus Albu or White Lupin,*). The commercial samples analyzed include, Ouzo and Baro Gin (supplied by National alcoholic beverages industry). White Horse Whisky and Stolichaniya Vodka were collected from supermarkets in Addis Ababa.

Tej and *Tella* were collected randomly from all sub-cities of Addis Ababa. A total of 20 samples for each fermented alcoholic beverages were collected. All the samples were collected using glass amber bottles and kept at 4°C. All samples were measured without sample pretreatment.

3.4 Analytical Methods

3.4.1 Method development

3.4.1.1 Selection of Spectral Range

In developing optical analytical method the first step is choosing a spectral range where the instrument response is selective to a specific analyte. Accordingly, the spectral range between 1720 – 1660 nm for the NIR and 1180 – 950 cm^{-1} for the MID region were selected. The absorptions in the NIR and MID regions are due to the O-H overtone and the C-O fundamental stretching vibrations, respectively. The spectral regions mentioned were used for the determination of ethanol and methanol in distilled alcoholic beverages.

For the determination of alcoholic content in fermented beverages the spectral range between 3020 – 2950 cm^{-1} was selected where C- H asymmetric stretching occurs.

3.4.1.2 Preparation of standard solutions

For preparing calibration curves standard solutions were prepared through dilution of 99.99% and 99.7% of ethanol and methanol, respectively.

The concentrations prepared were between 1-100% (*w/w*) for ethanol and 0.1-15% (*w/w*) for methanol.

3.4.1.3 Working and Linear Range Selection

For any quantitative method, it is necessary to determine the range of analyte concentrations over which the method is applied. This refers to the range of concentrations whereby the instrument response, i.e. absorbance can be attributed to the sample.

Working range defines the concentration range where there is functional relationship between absorbance and concentration, whereas the linear range refers to the range of concentration whereby the absorbance changes linearly with the concentration. For both techniques the working range and linear ranges were determined.

3.4.1.4 Calibration for FT-MIR and UV-NIR

For the working range, ethanol and methanol standards ranging from 1 to 99.99% (*w/w*) and 0.1 to 80% (*w/w*), respectively, were prepared.

3.4.2 Method Validation

For an analytical result to be acceptable for its intended purpose it must be sufficiently reliable so that any decision based on it can be taken with confidence. Thus, the method performance must be validated before it can be applied for the analysis of the samples. In the method validation of both techniques, the following validation parameters were considered.

3.4.2.1 Working range

The working ranges were selected after the standard solutions up to 100% (*w/w*) were measured. The selections of the ranges were done based on the band shift observed (NATA, 2012).

3.4.2.1 Linearity range

The linearity of an analytical procedure is its ability to induce a signal (response) that is directly proportional to the concentration of the given analytical parameter. For both techniques the linear ranges were extracted from the working ranges (NATA, 2012).

3.4.2.3 Limit of Detection (LOD) and Limit of Quantification (LOD)

Limit of detection is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantify under the stated conditions of the test whereas limit of quantification refers to the lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test. Independent sample blank water ($n>10$) was measured for the determination of both LOD and LOQ. The LOD was expressed as standard deviation of the blank value times three ($LOD = 3 \times SD$ blank) and the LOQ was expressed as standard deviation of the blank value times ten ($LOD = 10 \times SD$ blank) (NATA, 2012).

3.4.2.4 Repeatability

The repeatability test was done to check the precision of the developed method. Repeatability gives an indication of the short-term variation in measurement results and is typically used to estimate the likely difference between replicate measurement results obtained in a single batch of analysis. For repeatability test seven data were collected according to NATA guide line (NATA, 2012).

3.4.2.5 Recovery

It is one of the method validation parameter and is expressed as the % of the known added amount of analyte recovered from a spiked test item. This was done using spiking of the analyte standards in the real sample. For the method recovery test different concentrations of the standard solution were used (NATA, 2012). Recovery is calculated as:

$$\% \text{ recovery} = \frac{(\text{total analyte found} - \text{analyte originally present})}{\text{analyte added}} \text{ x } 100$$

3.4.3 Comparison of the methods with GC-FID

In addition to the above validation parameters, the accuracy of the proposed methods (using NIR and MID techniques) were compared with GC-FID method. The chromatographic conditions injection volume, oven temperature, injection port temperature; flow rate, detector temperature and column were optimized.

For both ethanol and methanol calibration curves were developed using the optimized conditions. The calibration curves were plotted using peak area versus concentrations. The calibration equations generated from the calibration curve fit were used for both ethanol and methanol analysis in the real samples.

3.5 Determination of pH

The pH of *Tella, Tej* and *Areke* were determined after collecting the sample using calibrated pH electrode at 25°C (AOAC, 2009b). The pH of the samples were analyzed immediately after the samples were collected. In all determinations the pH meter was calibrated before analysis using pH buffers of 4.0, 7.0 and 9.2.

3.6 Determination of titratable acidity

The titratable acidity of *Areke*, *Tella* and *Tej* was conducted potentiometrically with pH meter. Twenty-five milliliter (25 mL) sample was placed in to 250 mL conical flask, and then the potentiometric titrations were carried out using 0.1N NaOH as titrant. The prepared sodium hydroxide solution was standardized using potassium hydrogen phthalate before use (AOAC, 2009b).

$$\text{Titratable acidity}\left(\frac{\text{g}}{100\text{ mL}}\right)=\frac{\text{ml of NaOH x normality of NaOH x 75}}{\text{ml of the sample}}\text{x M}$$

Where: M - the conversion factor of the titratble acidity (M = 1 for tartaric acid, 0.8 for acetic acid).

3.7 Statistical Analysis

All results were presented as mean values ± standard deviation. Differences in mean values were determined by analysis of variance (ANOVA). Significance levels were obtained using Duncan test. Differences were considered statistically significant for p-values of less than 0.05. All analyses were done in triplicate. Microcal Origin Lab 6 and 8 was used for statistical analyses.

4. Results and Discussion

4.1 Method development

4.1.1 Selection of Frequency and Wavelength Ranges for the Distilled Alcoholic Beverage '*Areke*'

The mid and near infrared spectra of ethanol in water solution were recorded. The spectra obtained are presented in Figures 2a, 2b and 2c.

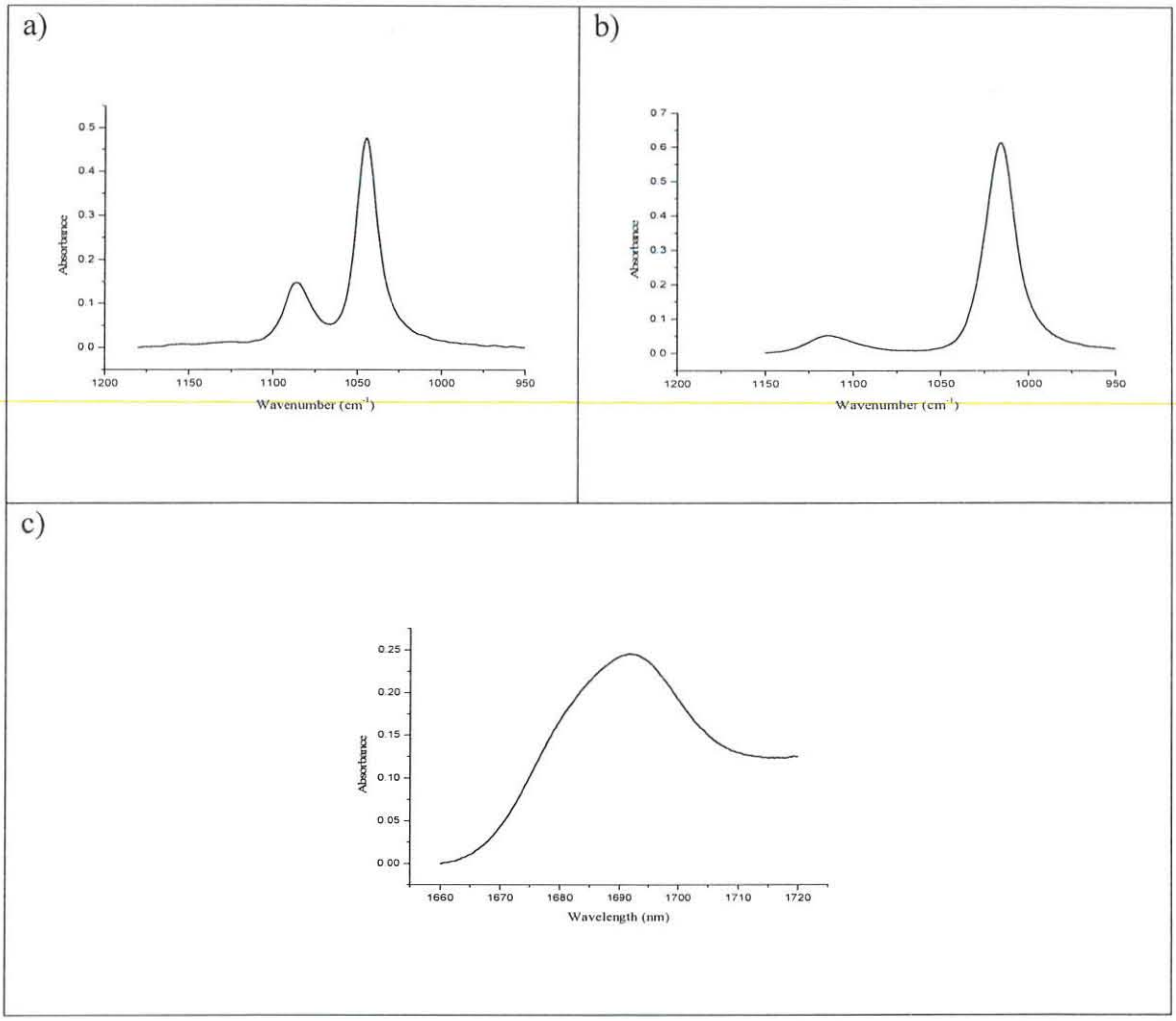


Figure 2: Mid IR spectra of (in the 1180 – 950 cm^{-1}) a) ethanol b) methanol and c) UV-NIR spectra of ethanol in the region 1660 – 1720 nm.

For both techniques (FT-MIR and UV-NIR) the spectral range, where the absorption of the analytes (ethanol and methanol) could be detected with less or no interference from the solvent matrix, were selected.

For the determination of methanol and ethanol in distilled alcoholic beverages in MIR, the spectral range between1180-950 $cm^{-1}$was chosen. This selection was based on the fact that in this spectral region the C-O stretching vibration absorption in alcohols occurs, whereas no absorption from the solvent (Pérez-Ponce & de La Guardia, 1998). In addition, the bands due to methanol and ethanol appear resolved allowing the selective determination of each of the alcohols. Therefore, this spectral region is suitable for the selective determination of methanol and ethanol in distilled beverages. However, the region is not appropriate for fermented beverages like *Tej*, since they contain sugars, which do absorb in the same spectral region (1180-950 cm^{-1}) (Figure 3).

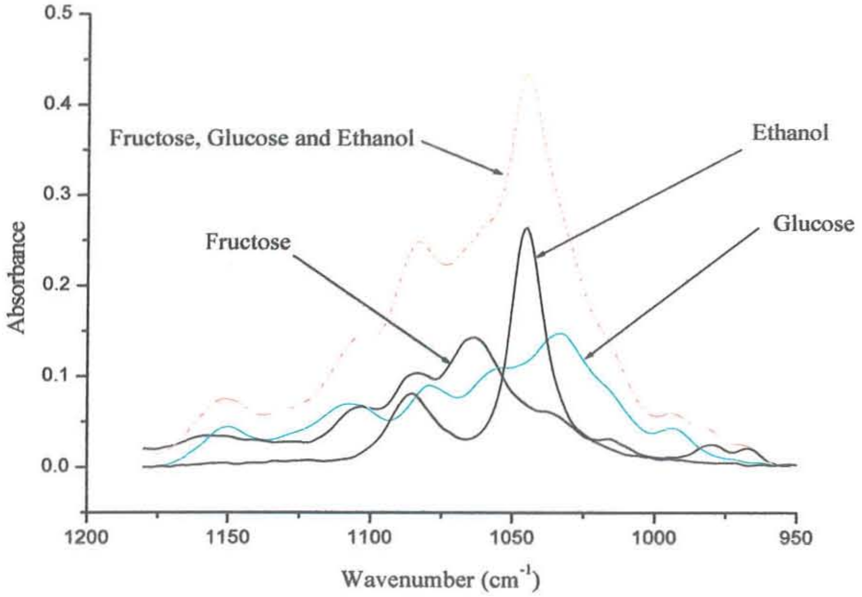


Figure 3: Spectra of Fructose, Glucose and ethanol in region1180 to 950 cm^{-1}.

In the NIR spectral range appear the overtone absorption and are usually broad. For alcohol determination the spectral range between 1720-1660 nm was selected. The absorption band is due to O-H overtone vibration and covers a broad spectral region. Though, broadness of the band makes it more suitable for quantitative determination, but not for selective determination of methanol and ethanol.

4.1.2 Selection of Frequency Ranges for Fermented Alcoholic Beverages

Fermented alcoholic beverages contain various ingredients. *Tella* consists of Barely (Hordeum vulgare L.) which serves as source of Bikil (malt); Enkuro; Gesho (Rhamnus prinoides L' Herit) leaves and stems chopped to small pieces; Kita (sour, unleavened bread made from Tef flour (Eragrostis tef), wheat, corn flours and *Tej* was made from Gesho (Rhamnus prinoides L' Herit) stems chopped to small pieces, honey and/or cane sugar (Sahle & Gashe, 1991).

The fermented alcoholic beverages, *Tella* and *Tej*, contain different ingredients including sugars such as fructose, glucose, sucrose and maltose. As a result, direct determination of ethanol from fermented alcoholic beverage *Tella* and *Tej* in the region 1180-950 cm^{-1} is not possible. Therefore, another spectral region between 3020–2950 cm^{-1} was selected for quantifying the level of ethanol in *Tella* and *Tej*.

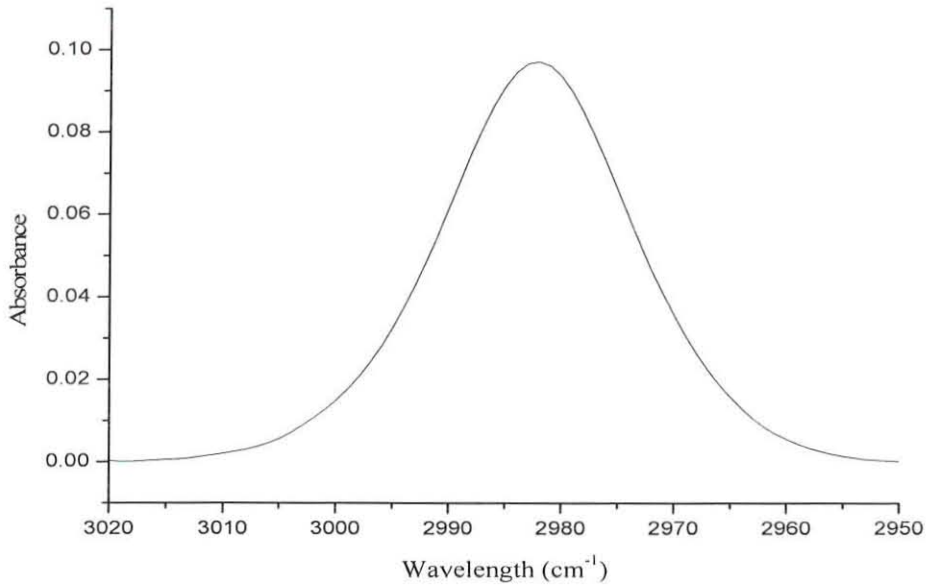


Figure 4: FT-MIR absorption spectra of ethanol in the region 3020- 2950 cm^{-1}

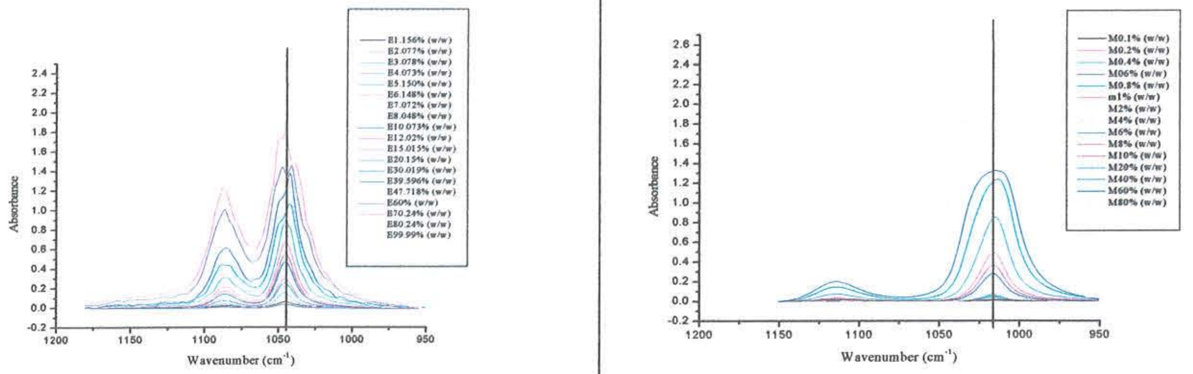
The spectral range selection was based on the absorption of C-H stretching vibration where absorption due to sugars does not occur. The spectra of ethanol in the region between 3020- 2950 cm^{-1} are presented in Figure 4.

4.1.3 Working Ranges for Distilled Alcoholic Beverages

For the determination of the working ranges, for both techniques (FT-MIR and UV-NIR)) series of ethanol and methanol standard solutions, between the concentration ranges 1 to 99.99 % (*w/w*) and 0.1 to 80 % (*w/w*) respectively, were prepared. The spectra are presented in Figure 5.

a) FT-MIR spectra of ethanol

b) FT-MIR spectra of methanol



c) UV-NIR spectra of ethanol

d) UV-NIR spectra of methanol

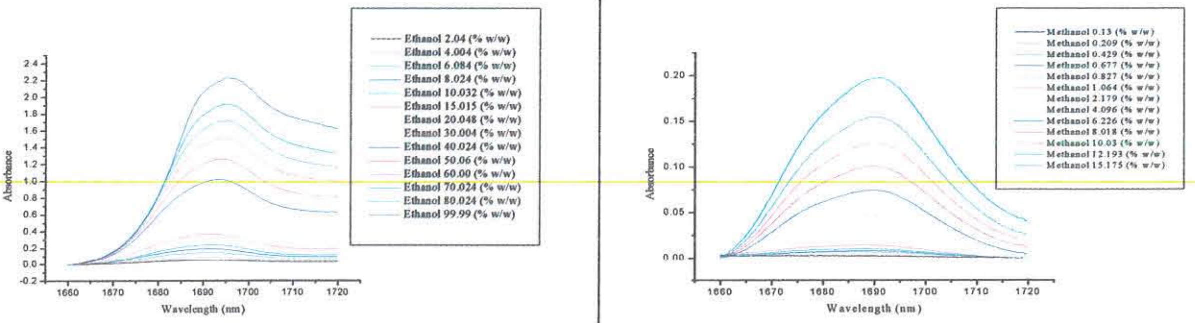


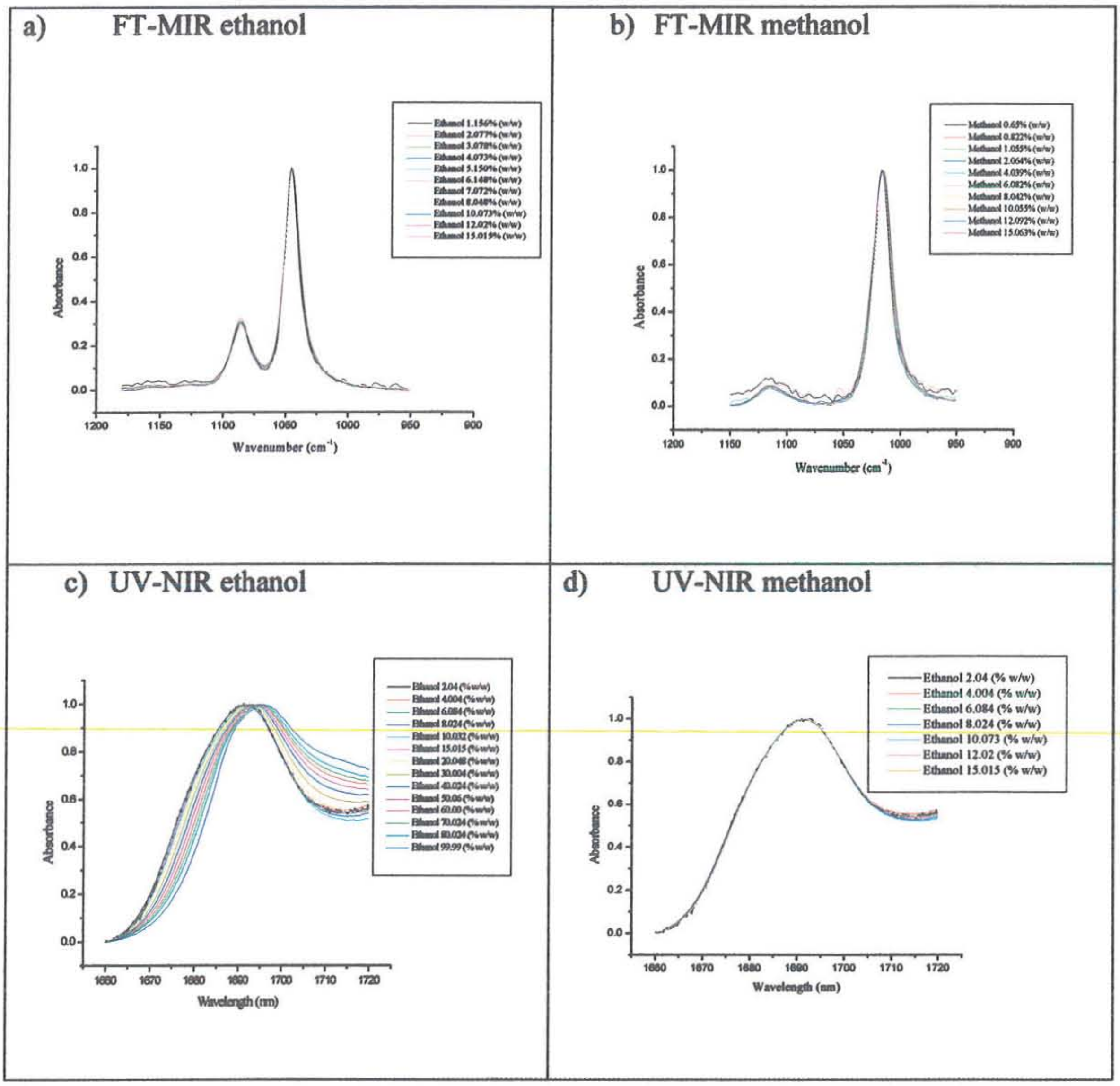
Figure 5: FT-MIR working range determination spectra for (a) ethanol (b) methanol and UV-NIR working range determination spectra for (c) ethanol (d) methanol for distilled alcoholic beverages.

Two closely resolved peaks at 1086 and 1045 cm^{-1} (Fig. 5), for ethanol and at 1125 and 1015 cm^{-1} for methanol were observed. The peaks are attributed to the C–O stretch frequencies. Interestingly, the values obtained in this work are different from that reported in the literature (Nagarajan et al., 2006) which may be due to the different measurement techniques employed.

The peaks at 1045 and 1015 cm^{-1} are the major peaks whereas peaks at 1086 and 1125 cm^{-1} are minor peaks of ethanol and methanol, respectively. The two major (intense) peaks were used for establishing the relationship between absorbance and concentration, and thus were used for the determination of the working ranges. The normalized spectra of ethanol and methanol for both techniques are presented in Figure 6.

Accordingly, 1- 15% (*w/w*) ethanol was prepared for the working range in FT-MIR for distilled alcoholic beverages and also for methanol 0.1- 15% (*w/w*) were prepared for distilled alcoholic beverages. For UV-NIR ethanol and methanol in the range of concentration between 1-99.99% (*w/w*) and1-15% (*w/w*) respectively, were prepared for distilled alcoholic beverages. The normalized spectra are presented in Figure 6.

The obtained result indicated that, the concentration ranges between 1-15% (*w/w*) and 0.1-15 % (*w/w*) respectively, for both ethanol and methanol no band shape change could be detected. Any change in the band shape can easily be confirmed by plotting together all the spectra measured for different concentrations. The normalized spectra of ethanol and methanol overlapped indicating no band shape change. When the concentration was increased beyond 15 % (*w/w*), in both techniques, the spectra showed clear band shape change, and is an indication of association. The attraction forces that exist between the associations is statistical and thus the spectra of undefined association is not reproducible, *i.e.* there is a defined relationship between the spectral response and the concentration and hence can't be used for quantification purpose.


a) FT-MIR ethanol

b) FT-MIR methanol

c) UV-NIR ethanol

d) UV-NIR methanol

Figure 6: FT-MIR normalized spectra of ethanol (a), methanol (b) UV-NIR normalized spectra for ethanol (c), and methanol (d) for distilled alcoholic beverages.

Therefore, 15% (*w/w*) was taken as the maximum concentration of the working ranges for both ethanol and methanol in the mid IR spectral range.

As discussed earlier, in the near IR spectral range, selective detection of ethanol and methanol is not possible, *i.e.* one must make sure there is no methanol in the sample before applying a method developed for ethanol determination and vice versa. For determining the working ranges the spectra of the whole concentration range (1-99.99% (*w/w*)) were recorded, and remarkably, no significant bandshape was observed. Therefore, the upper limit of the working range in UV-Vis technique is the same as the maximum concentration of pure ethanol; in this case it is 99.9% (*w/w*).

4.1.4 Working Range for fermented Alcoholic Beverages

The working range was determined only for ethanol, since, from the analysis, results to be showed later, no methanol concentration could be detected. Therefore, a series of ethanol standard solutions with concentrations between 1 and 30 % (*w/w*) were prepared. The spectra obtained and normalized are presented in Figures 7 and 8.

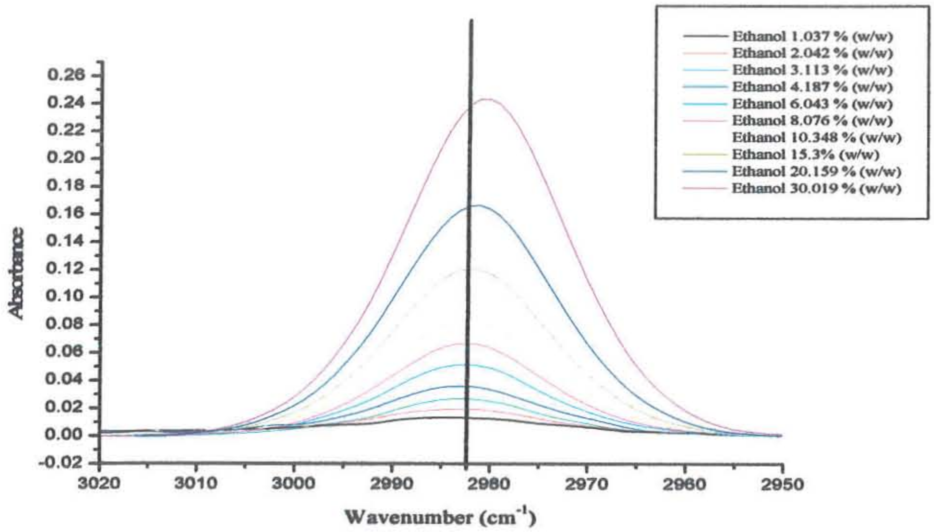


Figure 7: Spectra of ethanol for different concentration in the 3020-2950 cm^{-1}

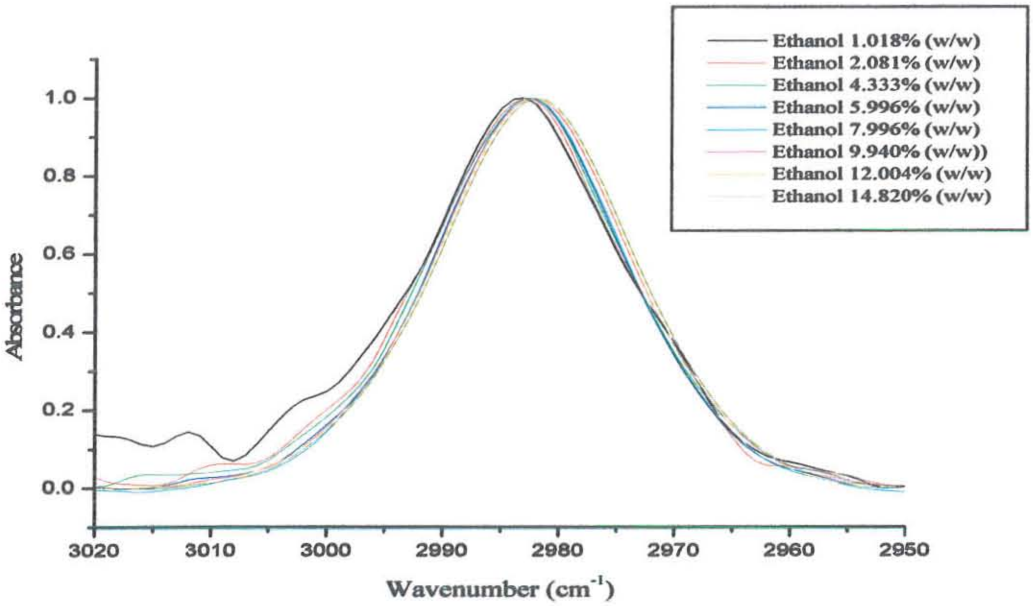


Figure 8: FT-MIR normalized spectra of ethanol in the region 3020-2950cm^{-1}

The spectra obtained showed no significant band shape change and therefore 15% (*w*/*w*) was established as the upper limit of the working range.

4.1.5 FT-MIR and UV-NIR Linearity Ranges for Distilled Alcoholic Beverages

From the working ranges, of ethanol and methanol up to 15% (*w/w*) in the region 1180 to 950 cm^{-1}, respectively and 99.99% (*w/w*) in UV-NIR the linear ranges were determined.

To select the linear range of ethanol and methanol in the region 1180 to 950 cm^{-1} using FT-MIR the working range of ethanol and methanol up to 15% (*w/w*) were used. The linear range selections for ethanol and methanol are presented in Figure 9 (a) and (b), respectively.

As showed in the Fig. 9 (a) and (b), the linear range of ethanol and methanol were determined by plotting the polynomial fit and the linear fit. The result obtained indicated that the point of intersection between the two graphs were 7% (*w/w*) for ethanol and 1% (*w/w*) for methanol.

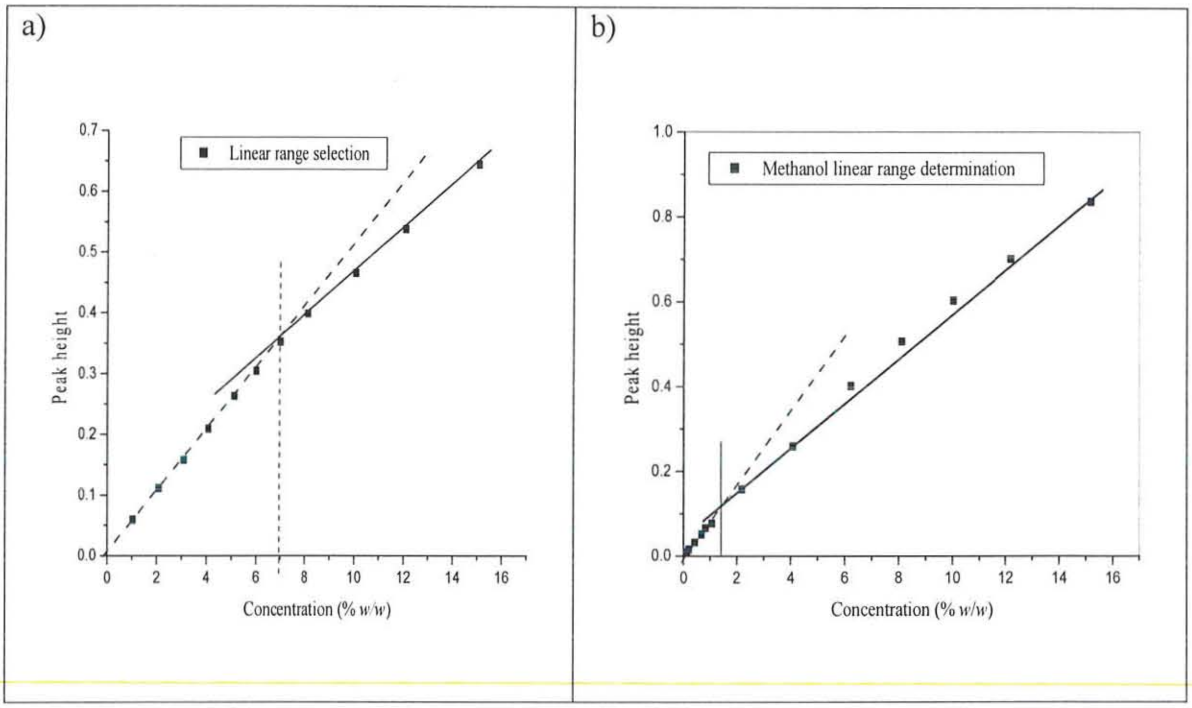


Figure 9: FT- MIR linear range selection a) ethanol and b) methanol in the region 1180 to 950 cm^{-1}.

The slope of the calibration curves of ethanol and methanol are greater than ten times of the y-intercept. This indicates the developed calibration equation with respect to the data obtained within the linearity range has small errors.

Similar procedures were carried out to select the linear range of ethanol in the region 1720 – 1660 nm using UV-NIR. A series of standard solutions of ethanol were prepared up to 100% (*w/w*). The linear range was extracted out from polynomial and linear fits and the curve are indicated in Figure 10.

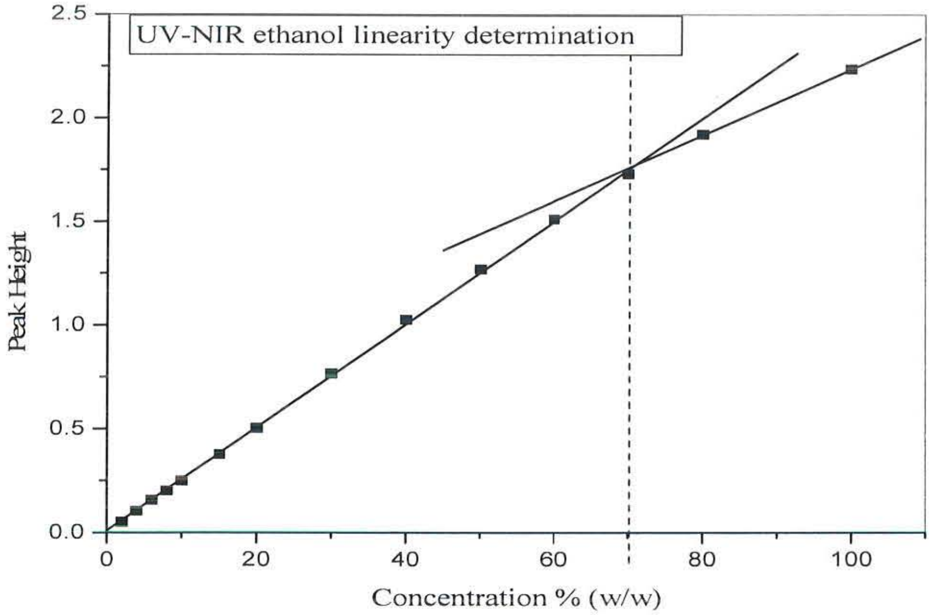


Figure 10: NIR ethanol linearity range selection in the region 1660 to 1720 nm

As indicated in Fig. 10, the linearity range selection curve of ethanol was up to 70% (*w/w*). However, for methanol the calibration curves at the beginning were linear. Therefore, further treatment was not conducted.

4.1.6 FT-MIR Ethanol Linear Range for Fermented Alcoholic Beverages

The linearity range of ethanol on the basis of C-H stretching in the region 3020 to 2950 cm^{-1} was determined. Since, the calibration graph initially was linear further treatment was not conducted. The linear calibration curves of ethanol are presented in Figure 11.

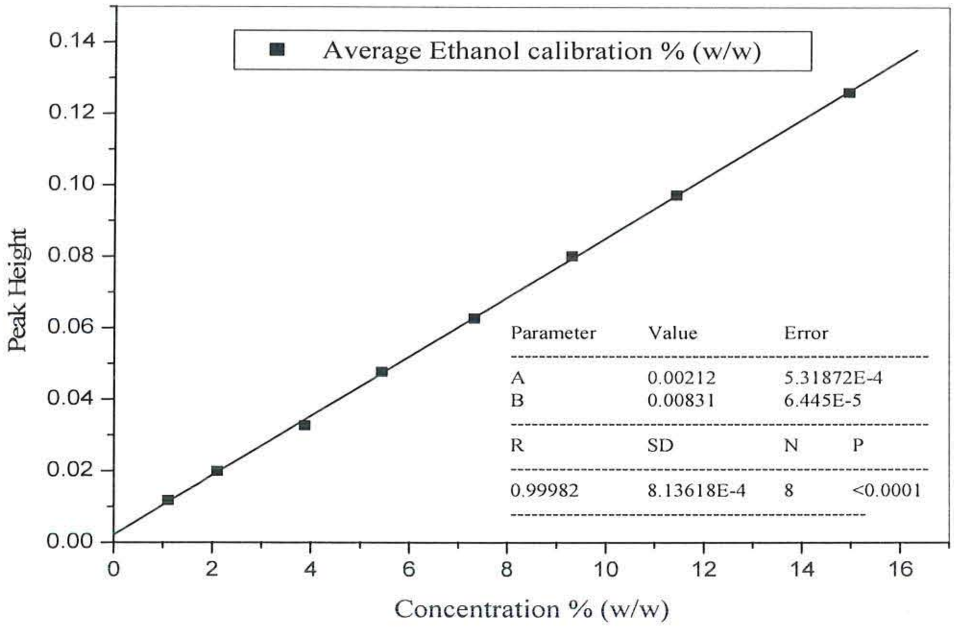


Figure 11: FT-MIR ethanol linearity range in the region 3020 to 2950 cm^{-1}

As indicated in the Fig 11, the linear range of ethanol in the region 3020 to 2950 cm^{-1} was similar to the working range.

Since, the slope of the calibration curve was ten times greater than the y- intercept. Ethanol up to 15% (*w/w*) was chosen as the linearity range on the basis of C-H stretching. Therefore, for fermented alcoholic beverages, '*Tella* and *Tej'*, it is possible to determine the level of ethanol without any sample dilutions.

4.1.7 FT-MIR Calibration Spectra and Curves for Distilled Alcoholic Beverages

For distilled alcoholic beverages, *Areke*, the calibration curve of ethanol and methanol in the region 1180 to 950cm^{-1} were constructed. To generate calibration curve a series of standard solutions of ethanol and methanol ranging between 1-15% (*w/w*) and 0.1- 15% (*w/w*) respectively, were prepared in triplicate. The average spectra are presented in Figure 12.

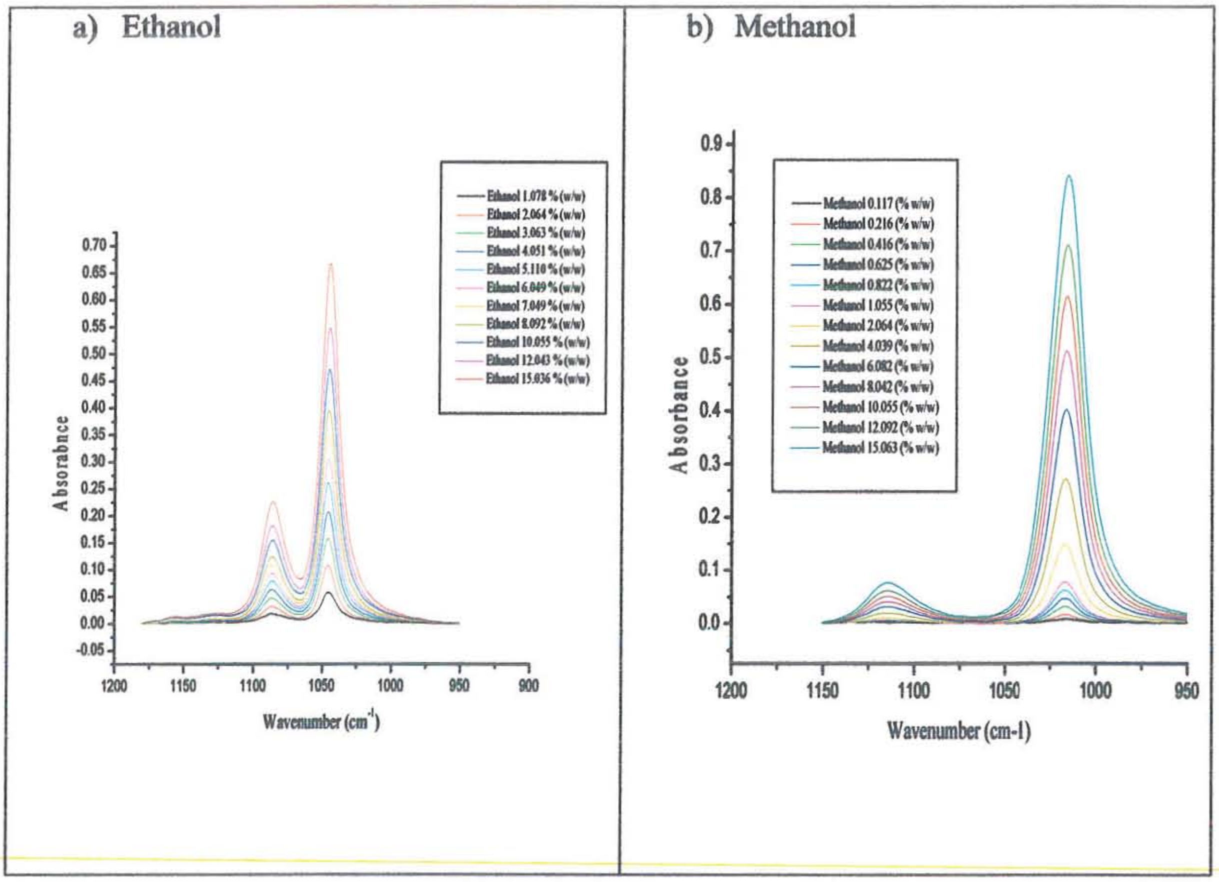


Figure 12: FT-MIR average calibration spectra of ethanol and methanol up to 15% (w/w) for distilled alcoholic beverages.

From Fig12, two distinctive absorbance peaks can be observed. For ethanol the first (major) peak is observed at 1045 cm^{-1} and the second (minor) peak is at 1085 cm^{-1}. The spectra of methanol have two peaks, the firs at 1025 cm^{-1} and the second one (major) peak at 1015 cm^{-1} as indicated in the calibration spectra. The absorbance for 0.1 % and 15% (*w/w*) methanol ranges are 0.00858 and 0.83658 nm respectively.

Accordingly, the calibration curve of ethanol and methanol were developed in triplicates. The average calibration curves of ethanol and methanol are presented in Figure 13.

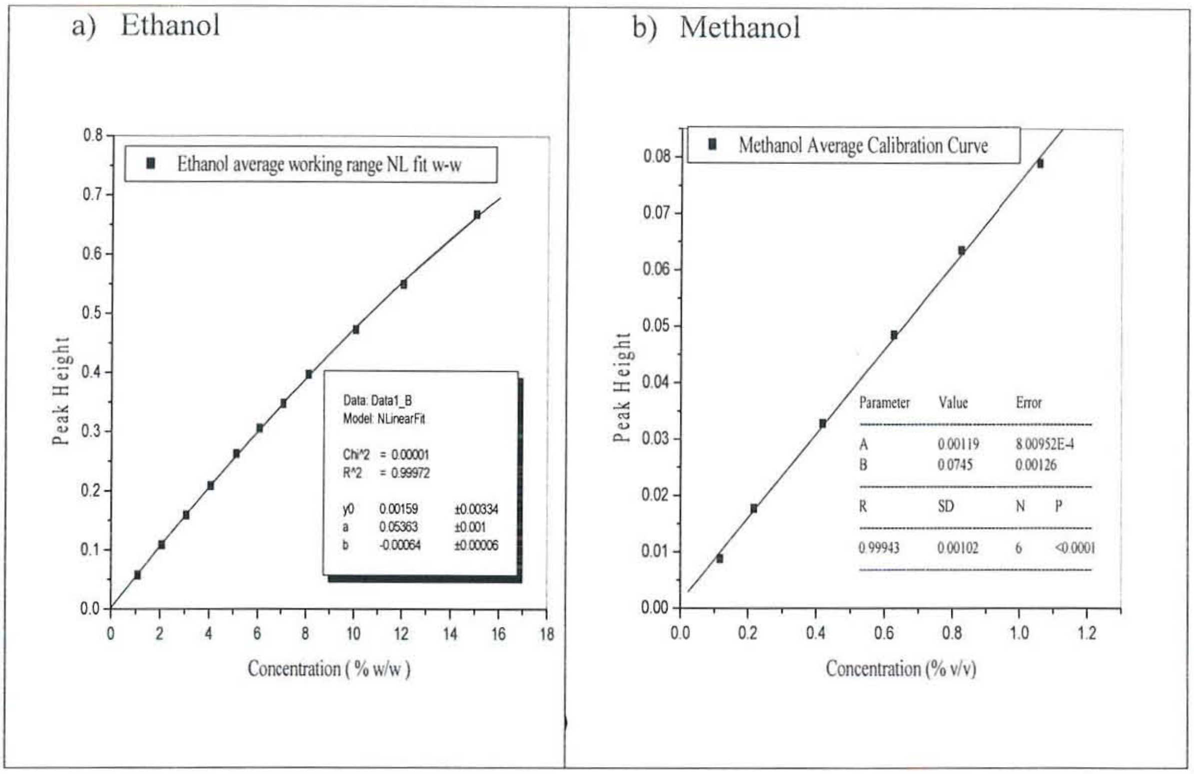
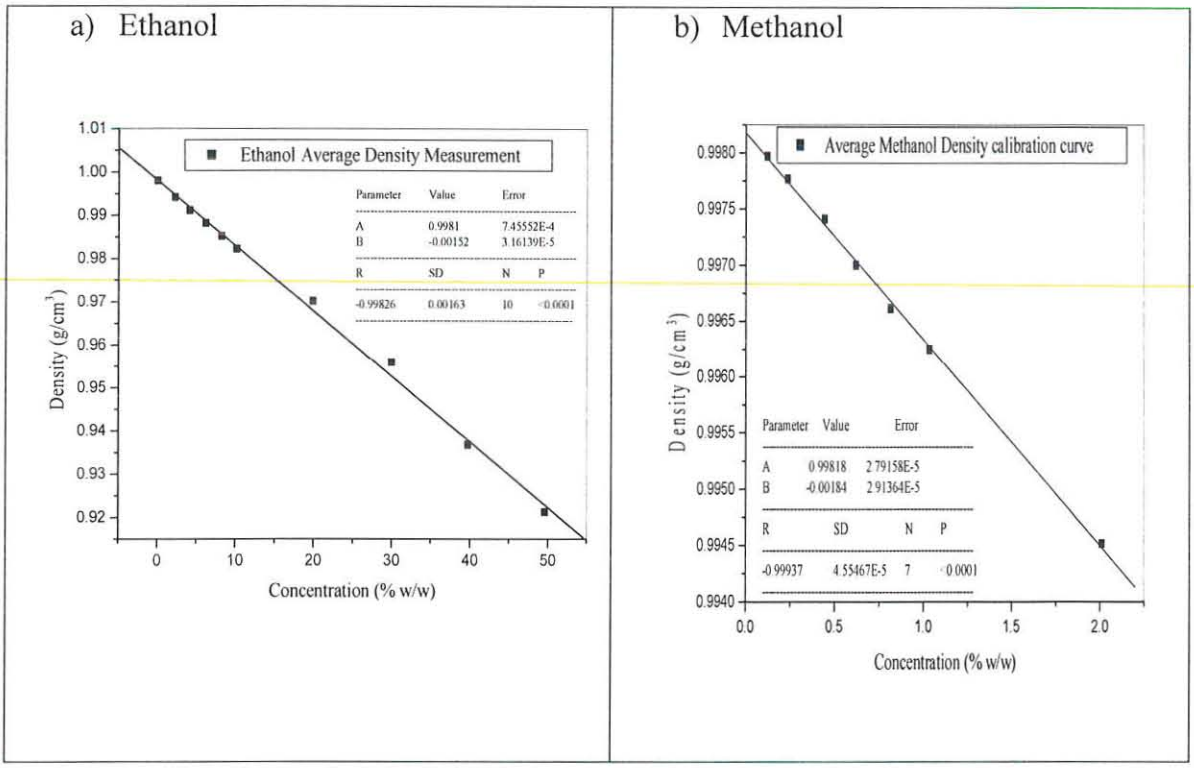


Figure 13: FT-MIR calibration curves for a) Ethanol and b) methanol for distilled alcoholic beverages.

As shown in Fig.13, the average calibration curves of ethanol up to 15% (*w/w*) were not linear. Thus, second ordered polynomial fit were selected for generating calibration curves. For methanol calibration curve up to 1% (*w/w*) was selected for the determination of methanol in distilled alcoholic beverages. The result obtained showed that the coefficients of determinations (r^2) of ethanol and methanol are greater than 0.999, respectively at p value < 0.0001. In addition, the slope of the calibration curves are greater than ten times of the y- intercept and the errors are very small for each ethanol and methanol Therefore, the proposed calibration curves of ethanol and methanol in the region 1180 to 950cm^{-1} can be used for alcohol determinations in distilled alcoholic beverages.

According to the European Commission the ethanol content of alcoholic beverages are reported in terms of % vol. Therefore, the % (*w/w*) versus peak height calibration curves of ethanol and methanol were converted to % (*v/v*) versus peak height after measuring the density of ethanol and methanol standard solutions using densitometer.

The density of the triplicate calibration standard solution of ethanol and methanol which are prepared in % (*w/w*) were measured and the density versus concentration in % (*w/w*) of ethanol and methanol were constructed and presented in Figure 14.


a) Ethanol

b) Methanol

Figure 14: Density versus % (w/w) a) ethanol and b) methanol.

From Fig.14, the negative relationship between density and concentrations of ethanol and methanol can be observed that. The negative sign indicates the graph is a decreasing function. The coefficients of determination of ethanol and methanol density calibration curve were 0.9981 and 0.99937, respectively.

From the density versus concentration % (*w/w*) equation of ethanol and methanol the calibration curves of ethanol and methanol in % (*v/v*) were constructed for distilled alcoholic beverages in the region 1180-950 cm^{-1}. The average calibration curves of ethanol and methanol in % (*v/v*) are presented in Figure 15.

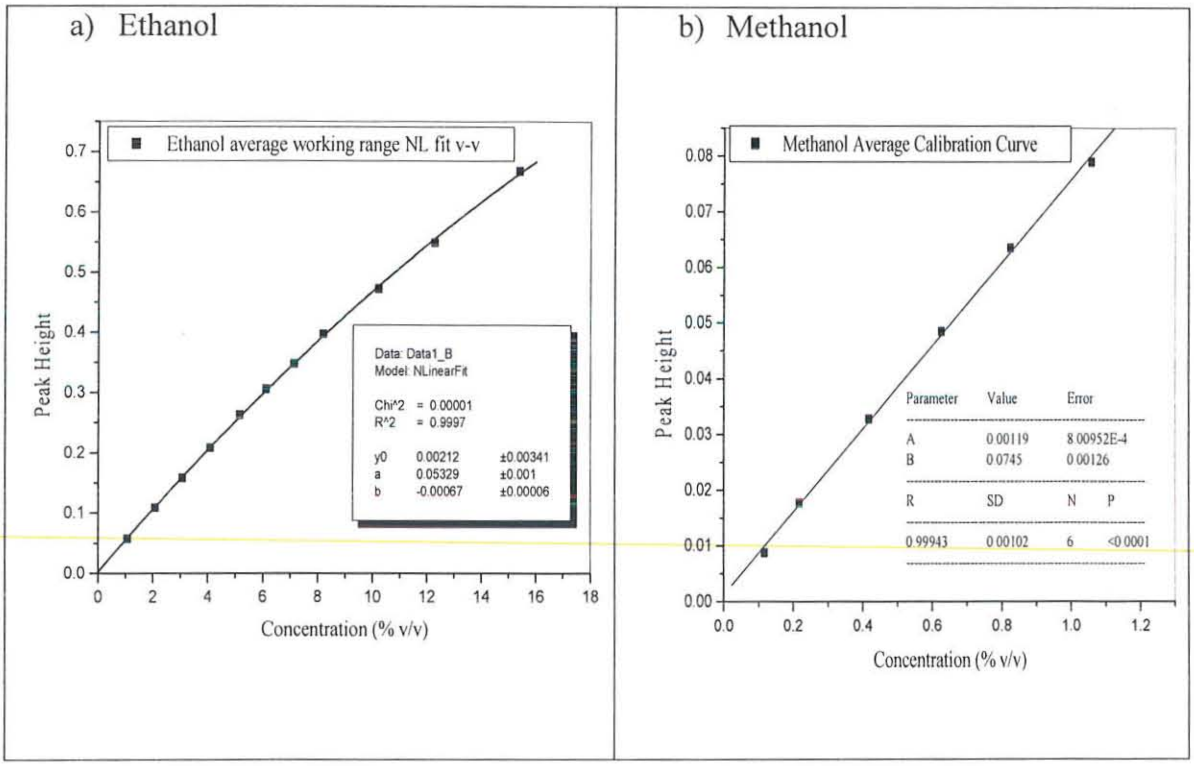


Figure 15: FT-MIR calibration curves of ethanol and methanol in % (v/v) for distilled alcoholic beverages.

As showed in Fig 15, the coefficient of determination (r^2) of ethanol and methanol were 0.9997 and 0.9994, respectively, at p value <0.0001.

To indicate the difference the calibration curves of ethanol in the different concentration units (% *w/w* and % *v/v*) are presented in Figure 16.

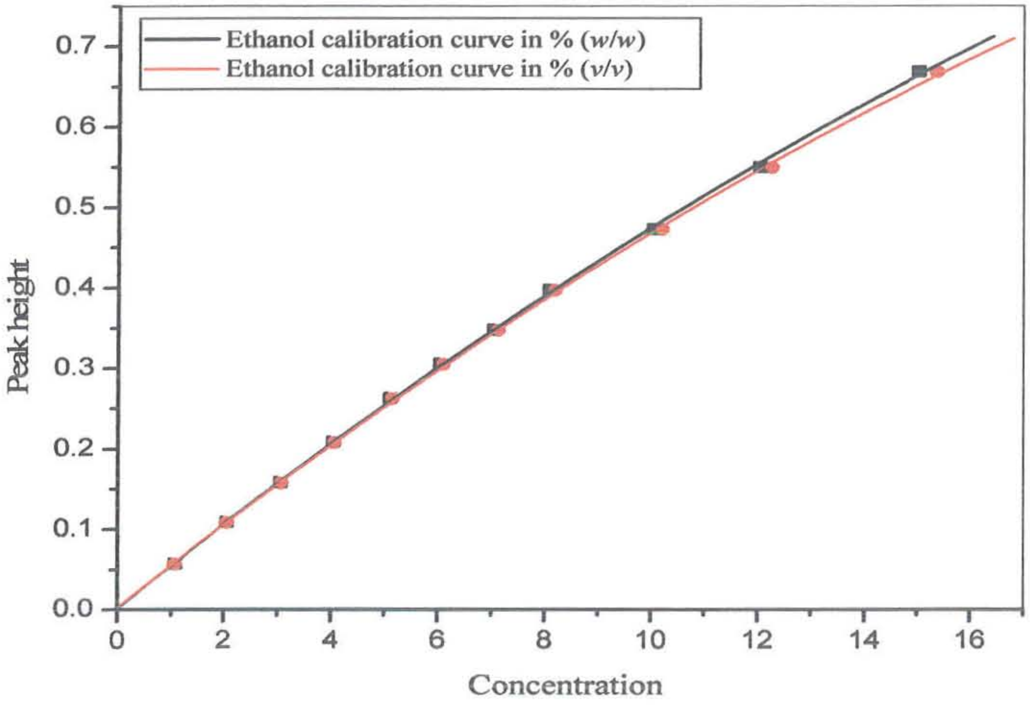


Figure 16: Comparison of % (*w*/w) and % (*v*/*v*) ethanol calibration curves.

From the above calibration curves there was a deviation between the % (*w*/*w*) and % (*v*/*v*) calibration equations. Therefore, to avoid errors all the measurements of ethanol and methanol were conducted based on of % (*w*/w) then using density of ethanol and methanol the % (*w*/*w*) are converted in to % (*v*/*v*).

4.1.8 UV-NIR Calibration Spectra and Curves for Distilled Alcoholic Beverages

For generating ethanol and methanol calibration curve using UV-NIR a series of standard solutions of ethanol and methanol in the concentration ranges 1- 50% (*w/w*) and 0.1-15% (*w/w*), respectively were prepared and analyzed in triplicates. The average calibration spectra of ethanol and methanol are indicated in Figure 17.

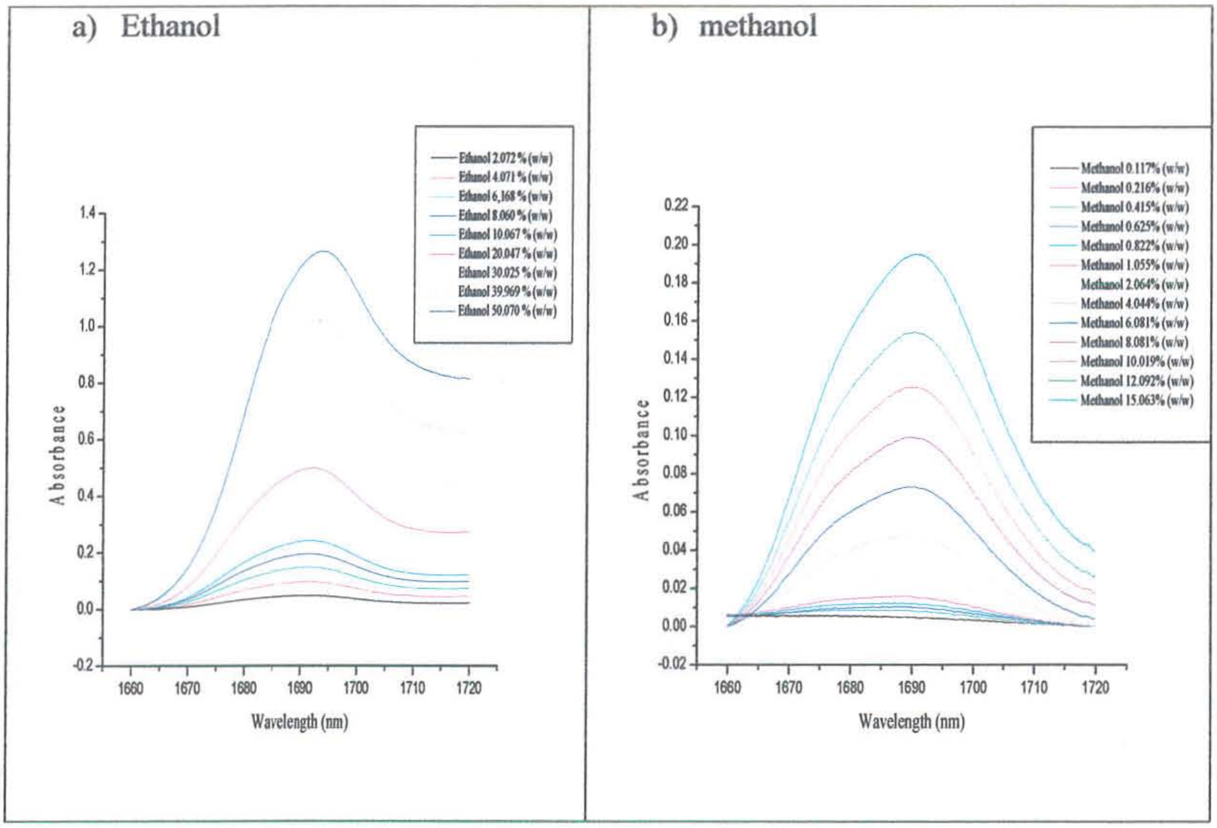


Figure 17: UV-NIR average calibration spectra for a) ethanol and methanol for distilled alcoholic beverages.

The result obtained indicated that both ethanol and methanol have only one peak. The maximum absorbance is at 1690 nm. Thus, with this technique it is impossible to differentiate whether the peak comes from ethanol or methanol.

The calibration curve of ethanol was generated based on peak height versus concentrations % (*w/w*). Accordingly, the concentrations of ethanol in % (*w/w*) were converted to % (*v/v*) using the density equation y = -0.00152x + 0.9981. The calibration graphs are indicated in Figure 18.

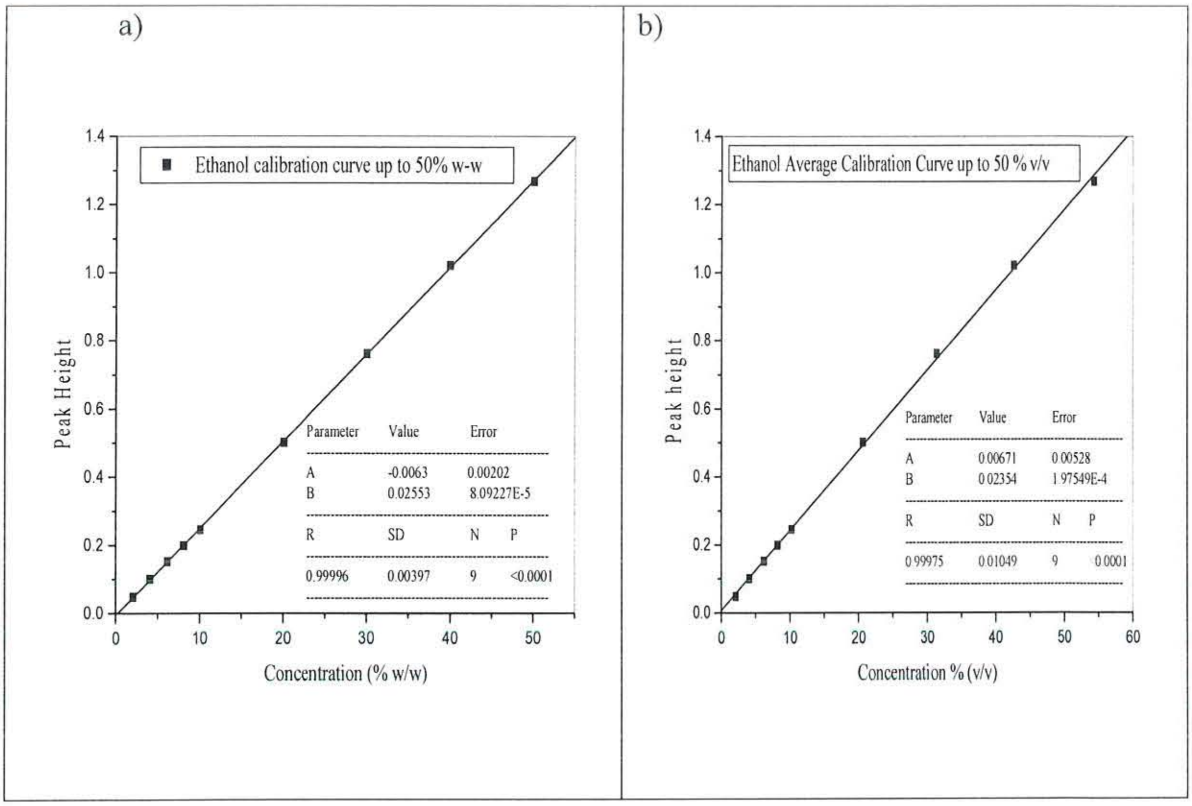


Figure 18: UV-NIR average calibration curves of ethanol a) in % (w/w) b) in % (v/v).

As shown above ethanol calibration curves are given in % (*w/w*) and (*v/v*) with r^2 values for both greater than 0.9997 and the slopes are 10 times greater than the y- intercept. Thus, the developed calibration curves were linear. Since, the level of alcohol in most sprits and traditional Ethiopian alcoholic beverage, *Areke*, are below 50 % (*v/v*). Based on the developed calibration curve it is possible to determine ethanol content without diluting the sample using UV-NIR.

To compare the two techniques methanol standards were prepared up to 15 % (*w/w*). Accordingly, the calibration curve was plotted by using peak height versus concentration of methanol % (*w/w*), then the % (*w/w*) was converted to % (*v/v*) using the methanol density equation (y = -0.00184x + 0.99818). The calibration curves are presented in Figure 19.

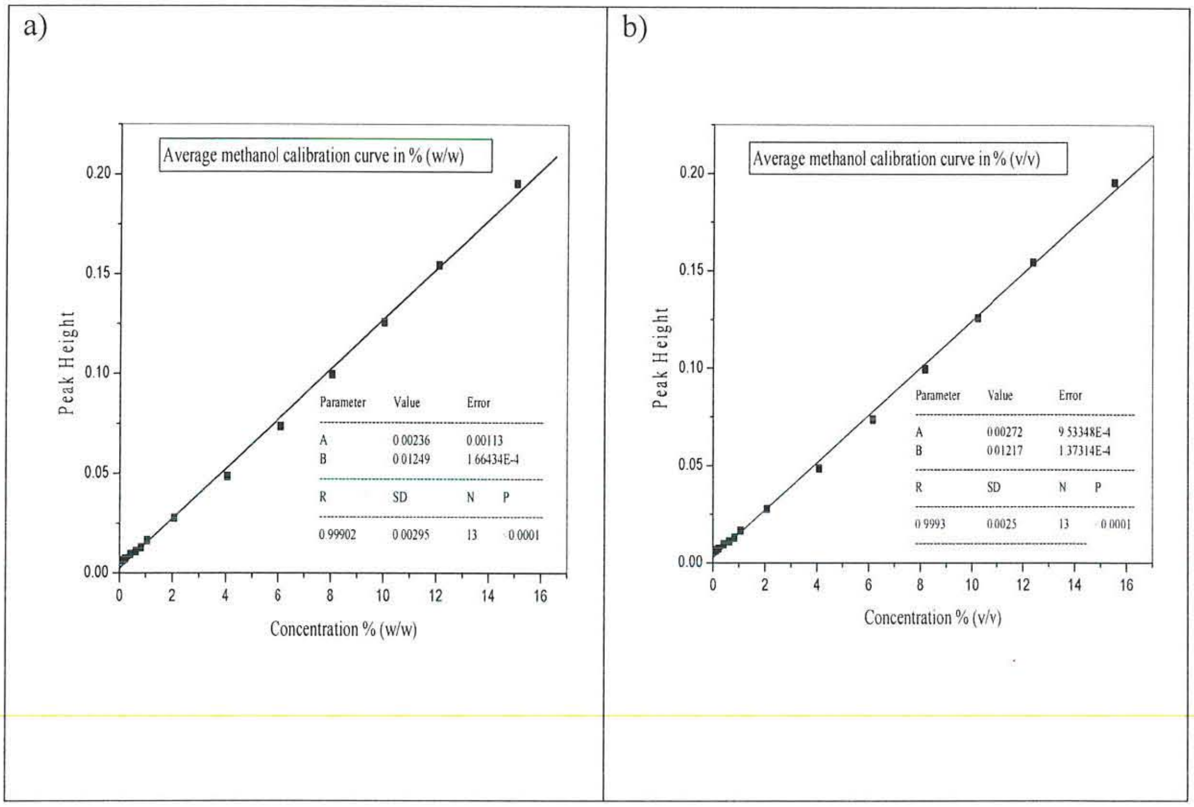


Figure 19: UV-NIR methanol calibration curves in a) % (*w/w*) and b) % (*v/v*).

The result obtained indicated that both the % (*w/w*) and % (*v/v*) have coefficient of determination greater than 0.999 and the slopes are greater than 10 times the y- intercept. However, in UV-NIR the absorbance of both ethanol and methanol are at the same wavelength, hence the determination of methanol in the presence of ethanol using this technique was impossible.

4.1.9 Calibration Spectra and Curves for Fermented Alcoholic Beverages

Frequency range between 3020 – 2950 cm^{-1} was selected on the basis of C-H stretching of ethanol for fermented alcoholic beverages, *Tella* and *Tej*. The calibration curves of ethanol ranging from 1-15% (*w/w*) in the selected region were developed in triplicates. For constructing the spectra of ethanol tangential base line fit were used using microcal origin 6. The average calibration spectra and curves in % (*w/w*) and % (*v/v*) of ethanol based on C-H stretching absorbance are presented in Figure 20 and 21, respectively.

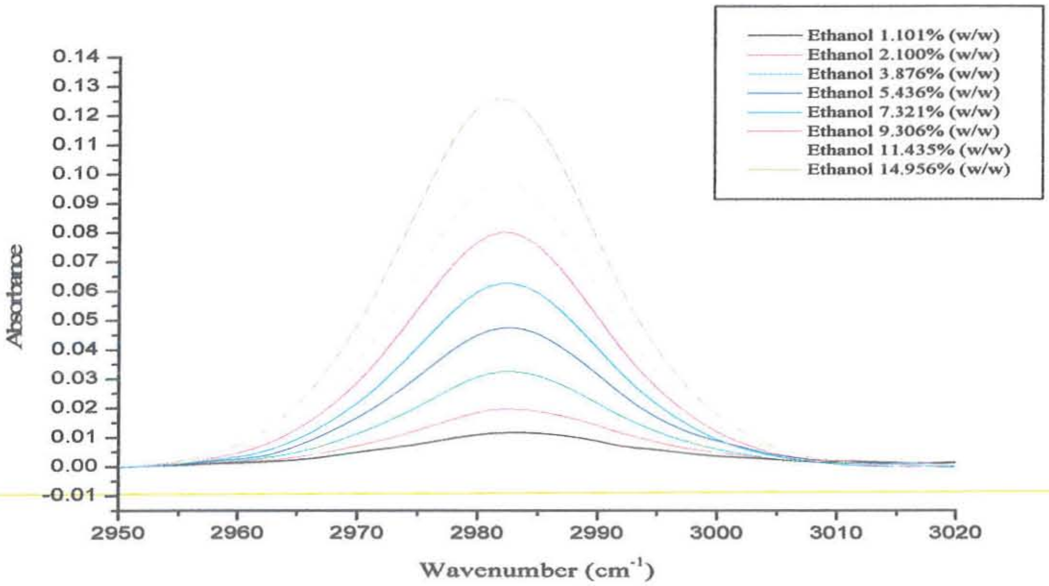


Figure 20: FT-MIR average ethanol calibration spectra for fermented alcoholic beverages, *Tella* and *Tej*.

The result obtained indicates that the absorption band of ethanol due to C-H stretching in the selected regions has only one peak. The maximum peak was obtained at 2982 cm^{-1}.

The calibration curves were established by plotting peak height versus concentration (% (*w/w*) and % (*v/v*)). As showed in figure 21 the calibration curves were linear. For both % (*w/w*) and % (*v/v*) the coefficients of determination were 0.9996 and 0.9998 respectively at p – value < 0.0001.

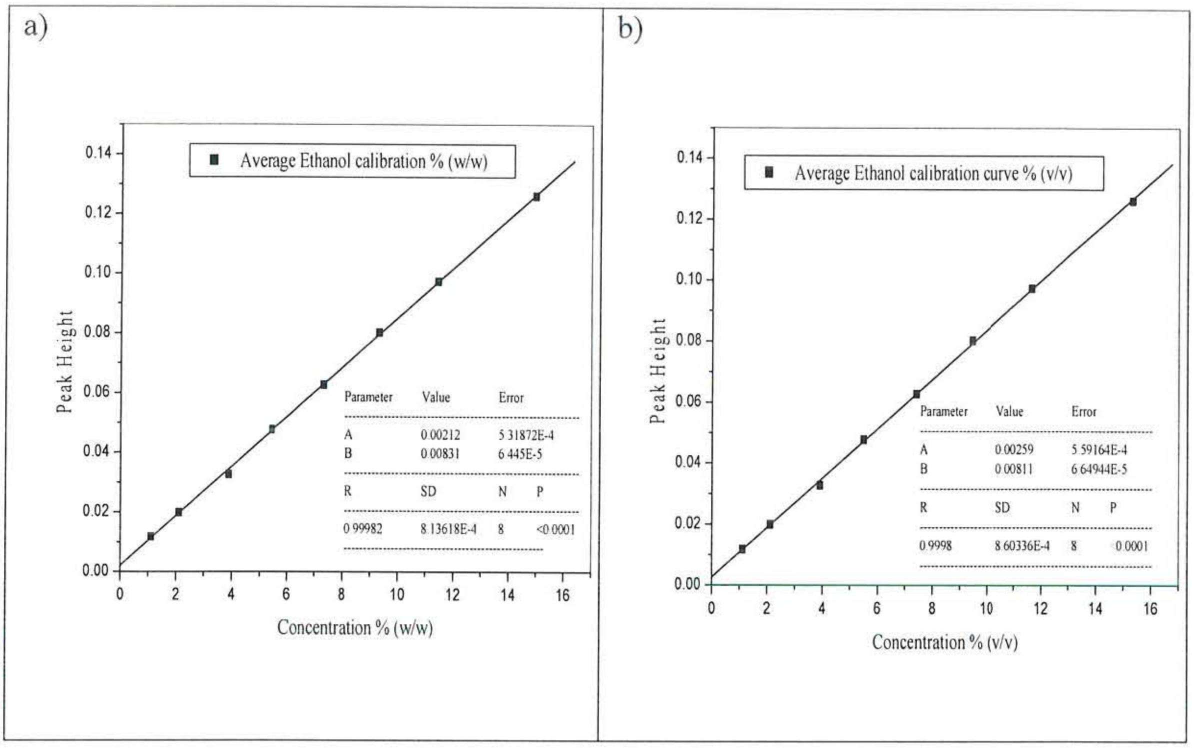


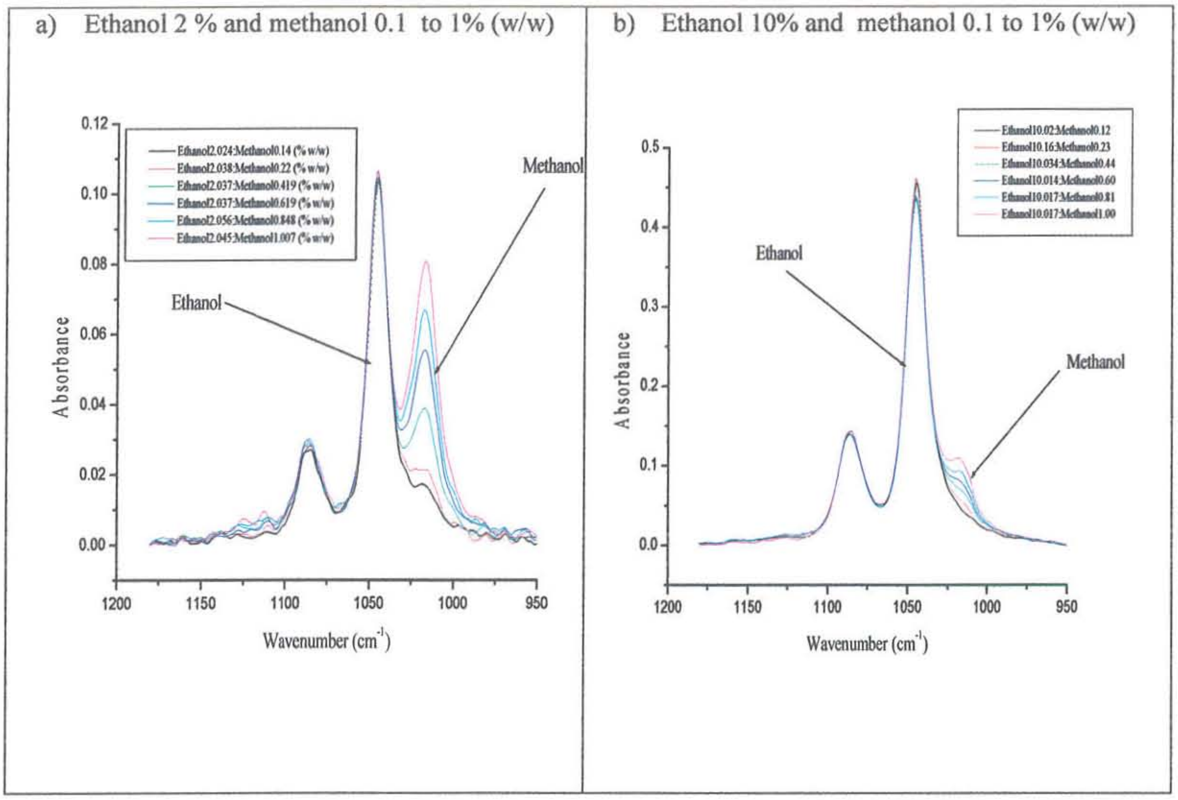
Figure 21: FT-MIR ethanol calibration curve a) % (w/w) and b) % (v/v).for fermented alcoholic beverages, *Tella* and *Tej*.

Calibration equation $y = 0.00811x + 0.00259$ were used for the determination of ethanol content in fermented alcoholic beverages *Tella* and *Tej* using FT-MIR in the spectral ranges between 3020 to 2950 cm^{-1} based on C-H stretching absorption bands .

4.2 Mixture of ethanol and methanol spectra comparisons

4.2.1 FT-MIR mixture spectra

The mixture spectra were determined in order to analyze the presence of methanol in the sample for both quantitative and qualitative purposes. Thus, mixtures of ethanol and methanol in different concentration ranges were taken. Mixture spectra of 2 and 10 % (*w/w*) ethanol with 0.1 to 1% (*w/w*) methanol respectively, are presented in Figure 22.



a) Ethanol 2 % and methanol 0.1 to 1% (w/w)

b) Ethanol 10% and methanol 0.1 to 1% (w/w)

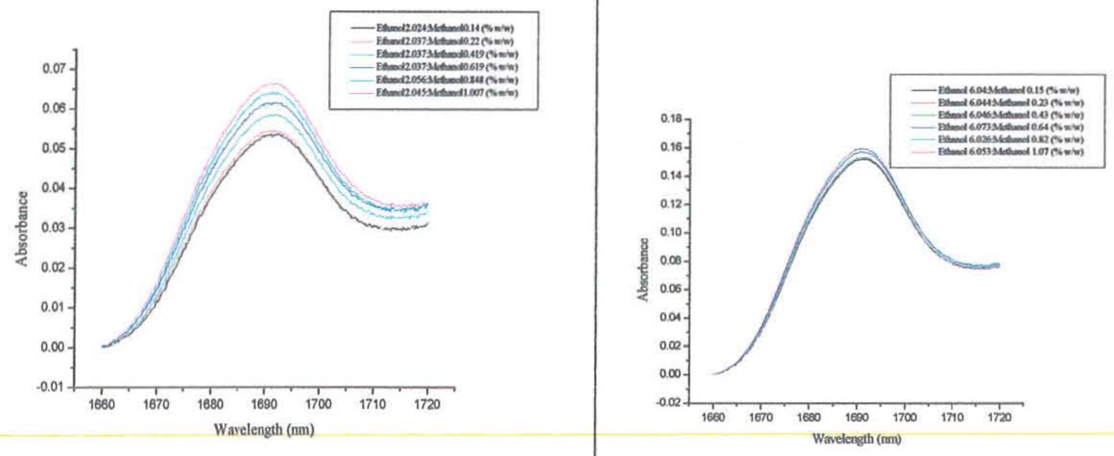
Figure 22: FT-MIR spectra of a) Ethanol 2 % and methanol 0.1 to 1% (w/w) and b) ethanol 10% and methanol 0.1 to 1% (w/w).

In the above FT-MIR mixture of ethanol and methanol four peaks are observed, two from ethanol at 1086 and 1045 cm^{-1} (the two middle peaks) and the remaining two from methanol at 1015 and 1125 cm^{-1} (the two extremities) (Figure 22). The major peak of ethanol is observed at higher frequency than methanol.

Two closely resolved peaks, obsereved at 1086 and 1045 cm^{-1}, are attributed to the C–O stretch of ethanol and two peaks at 1125 and 1015 cm^{-1} are due to the C–O stretch of methanol in the region 1180-950cm^{-1} using FT-MIR. This is different from the reported values in the literature. (Nagarajan et al., 2006).

4.2.2 UV-NIR mixture spectra

Similarly, the performance of UV-NIR in the selected region was checked. Thus, mixture of ethanol 2% and 6% (*w/w*) with 0.1 to 2% (*w/w*) methanol, respectively, were prepared and the spectra were presented in Figure 23.


a) ethanol 2 % and methanol 0.1 to 1% (*w/w*)

b) ethanol 6 % and methanol 0.1 to 1% (*w/w*)

Figure 23: UV-NIR spectra of a) ethanol 2 % and methanol 0.1 to 1% (*w/w*) and b) ethanol 10% and methanol 0.1 to 1% (*w/w*).

The result obtained in UV-NIR spectra showed an increment in absorbance as the composition of ethanol and methanol concentrations increased and only one peak was observed. In addition, a single effect on one component has been observed as an effect of the total. This is because both of them involve in the absorbance of O-H stretching absorption bands.

Therefore, FT-MIR technique in the region 1180-950cm^{-1} can be used for simultaneous determination of ethanol and methanol.

4.3 Comparison of pure, and mixtures of ethanol and methanol using FT-MIR

Pure ethanol and methanol having equal concentrations to the composition of the mixtures were compared. The spectra of pure ethanol, methanol and the mixture are presented in Figure 24.

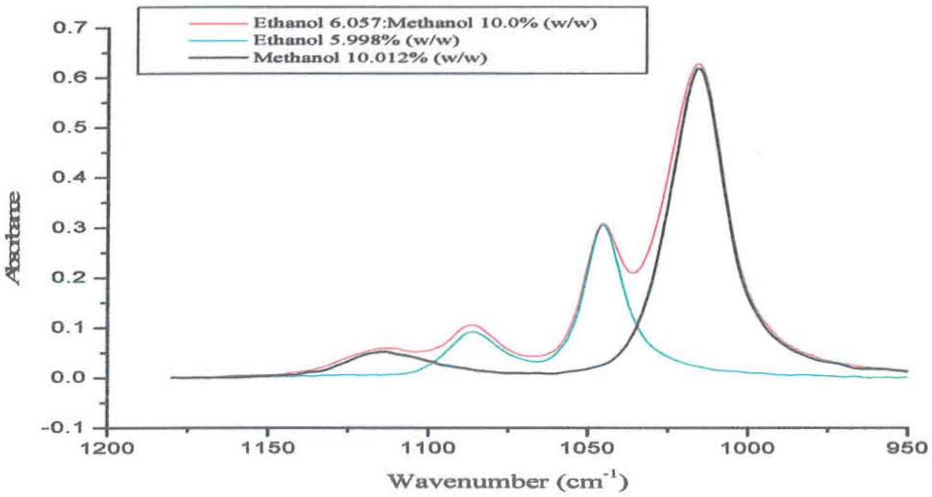


Figure 24: FT-MIR comparison spectra of the mixture with pure ethanol and methanol.

The result obtained in the spectra of pure ethanol and methanol having equal concentrations to the composition of the mixtures showed no significant difference in the amount. The overlapped peaks of the mixtures with equal concentrations of pure ethanol and methanol respectively showed no interference between them. Therefore, the FT-MIR techniques can be used for the direct determinations of ethanol and methanol in distilled alcoholic beverages.

4.4 Method Validation

According to NATA, (2012) method validation can be defined as the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. Thus, it is necessary to evaluate the method's performance capabilities. Therefore for this study the following validation parameters were considered.

4.4.1 Limit of Detection and Quantification

In this study the blank solution, water, was used. Independent sample blank (n >10) were measured for both LOD and LOQ. The LOD was expressed as standard deviation of the blank value times three (LOD = 3 × SD blank) and the LOQ was expressed as standard deviation of the blank value times ten (LOD = 10 × SD blank). The results obtained for both ethanol and methanol for the proposed techniques for distilled and fermented alcoholic beverages with respect to their frequency and wavenumber ranges are presented in Table 1.

The LOD and LOQ using FT-MIR for distilled alcoholic beverages in the region 1180-950cm^{-1} were 0.025 and 0.082, respectively, for ethanol, and 0.007 and 0.024, respectively, for methanol. For fermented alcoholic beverages *Tella* and *Tej* the LOD and LOQ of ethanol in the region 3020-2950cm^{-1} based on C-H stretching absorbance was 0.08 and 0.27 respectively. However, for methanol in the region 3020 – 2950 cm^{-1} , since the absorbance of methanol was affected by sugars, the LOD and LOQ were not determined.

Table 1: limit of detection and quantification of ethanol and methanol for distilled and fermented alcoholic beverages.

Method	**Wavenumber or wavelength range**	**Ethanol**		**Methanol**	
		LOD	**LOQ**	**LOD**	**LOQ**
		% (v/v)	**% (v/v)**	**% (v/v)**	**% (v/v)**
FT-MIR	1180 – 950cm^{-1}	0.025	0.082	0.007	0.024
FT-MIR	3020 – 2950cm^{-1}	0.080	0.270	*	*
NIR	1660 – 1720 nm	0.019	0.065	0.038	0.125

* *Not available*

For UV- NIR in the spectral range between 1720-1660 nm for distilled alcoholic beverages the result obtained indicated that the LOD and LOQ for ethanol were 0.019 and 0.065, respectively, where as for methanol the LOD and LOQ were 0.038 and 0.125, respectively. The linear ranges proposed for the methods are above the LOD and LOQ. Therefore, the developed spectroscopic techniques can detect the concentration of ethanol and methanol at low levels.

4.4.2 Repeatability

To verify the precision of the developed method repeatability test was performed for each technique. For distilled alcoholic beverages the repeatability of the methods were checked by 2 and 6 % (*w/w*) ethanol and 0.6 and 1.0 % (*w/w*) methanol within linearity range of FT-MIR in the region 1180 to 950cm^{-1}. For UV-NIR 2 and 12% (*w/w*) ethanol and 1.04 and 8.018 % (*w/w*) methanol were selected randomly. Moreover, for fermented alcoholic beverages the repeatability of the method were checked by real sample of *Tella* and *Tej* selected randomly based on color difference. For each technique seven (independent) samples were analyzed (NATA, 2012). The repeatability deviations of the methods are indicated in Table 2.

The result obtained for distilled alcoholic beverages using FT-MIR in the region 1180-950cm^{-1} based on C-O stretching showed the repeatability deviations for ethanol and methanol were 0.002-0.0046 and 0.0006-0.001 respectively. Similarly, in UV-NIR the repeatability deviations for ethanol and methanol were 0.0005-0.008 and 0.0005-0.001 respectively. For Fermented alcoholic beverages, *Tella* and *Tej*, the repeatability deviation of ethanol was 0.001-0.002.

Table 2: Repeatability of ethanol and methanol for distilled and fermented alcoholic beverages.

Method	Wavenumber or frequency ranges	Ethanol repeatability deviation	Methanol repeatability deviation
FT-MIR	1180 – 950cm^{-1}	0.002 - 0.005	0.0006 - 0.001
	3020 –2950cm^{-1}	0.001 - 0.002	**
NIR	1660 – 1720 nm	0.0005 - 0.008	0.0005 - 0.001

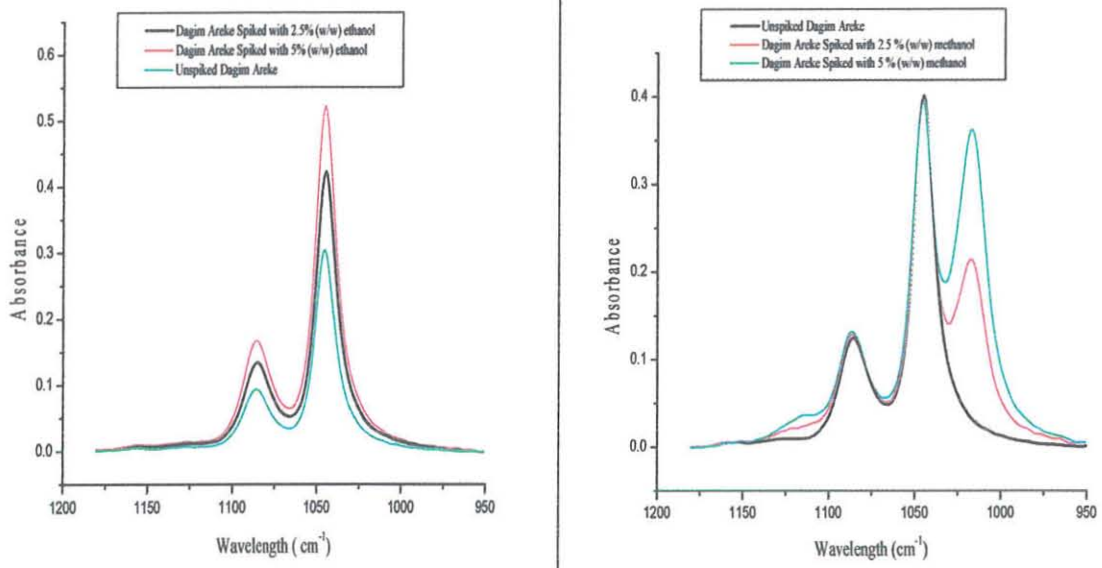
** *Not available*

In general, repeatability deviations obtained for both techniques were very small which implies that the short term variations between the replicate measurements are insignificant. Therefore, the developed method is highly precise.

4.4.3 Recovery Test

The accuracy of the developed methods was checked using unspiked samples of *Areke, Tella* and *Tej* and with known concentration of ethanol and methanol spiked samples. The spiked concentration of ethanol and methanol were 2.5% and 5% (*w/w*), respectively, for both techniques of FT-MIR and UV-NIR in the proposed regions,

For distilled alcoholic beverages the randomly sampled *Arekes* was *Dagim Areke* from Dembecha. However, for *Tella* and *Tej* two different varieties for each were considered based on their color differences and analyzed by using FT-MIR and UV-NIR in triplicates. Spectra ethanol and methanol for the proposed methods are indicated in Figure 25 and 26, respectively.



a) FT-MIR *Areke* ethanol recovery

b) FT-MIR *Areke* methanol recovery

a) UV-NIR *Areke* ethanol recovery

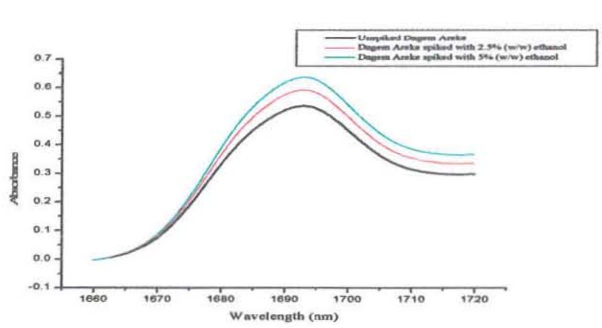
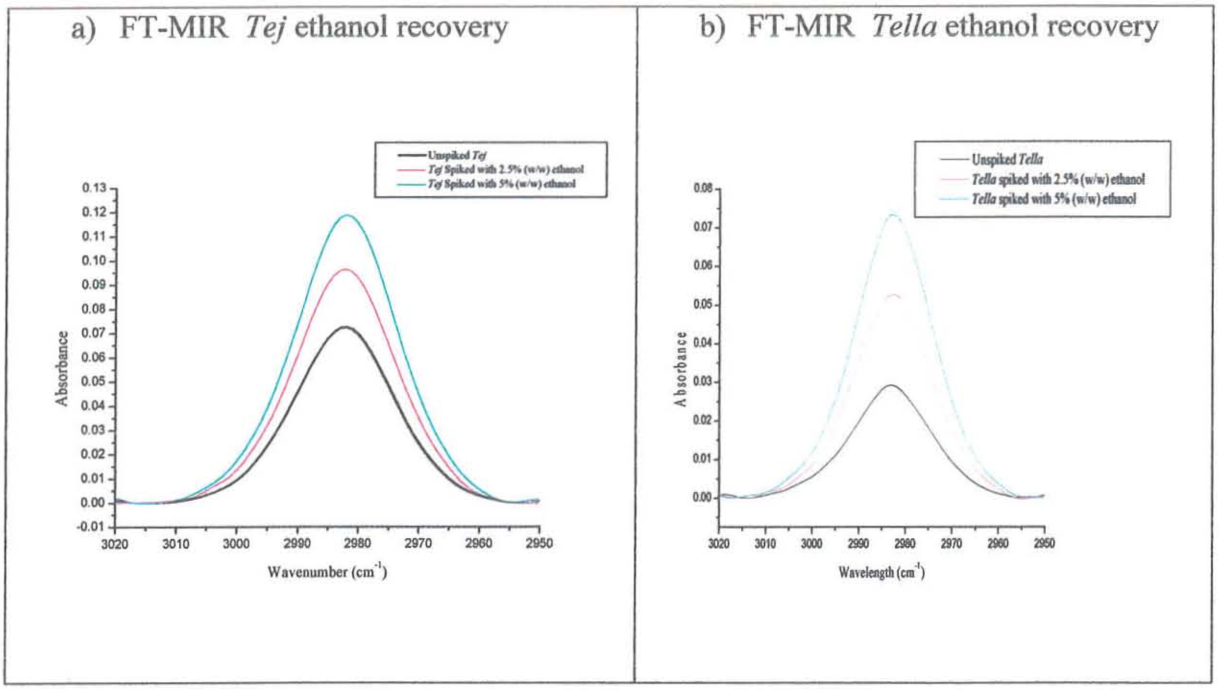


Figure 25: FT-MIR recovery spectra for *Areke* a) ethanol b) methanol c) UV-NIR ethanol recovery spectra of *Areke.*


a) FT-MIR *Tej* ethanol recovery

b) FT-MIR *Tella* ethanol recovery

Figure 26: FT-MIR ethanol recovery spectra a) *Tej* and b) *Tella.*

In the above recovery spectra of ethanol and methanol the result obtained indicated that as the spiked concentration increases the absorbance also increases proportionally. Moreover, the methanol recovery test in FT- MIR in the region 1180 – 950 cm^{-1} showed the absence of methanol in the unspiked samples. The % recovery ranges are indicated in Table 3.

The recovery result obtained for ethanol in distilled alcoholic beverages was between 97.52-101.65 and for *Tella* and *Tej* samples it was between 99.43-102.83 and 100.59-102.44% (*v/v*), respectively, using FT-MIR in the respective frequency ranges. Recovery of methanol for distilled alcoholic beverages, *Areke* was 98.42-100.67 % (*v/v*).

Ethanol and methanol recovery obtained for *Areke* samples using UV-NIR ranges were 99.08-104.96 and 99.41-100.94% (*v/v*), respectively.

Table 3: Recovery of ethanol and methanol in the developed methods

Method	Wave number or frequency ranges	Type of Beverages	Ethanol % Recovery	Methanol % recovery
FT-MIR	1180 – 950cm^{-1}	*Areke*	97.52 – 101.65	98.42 – 100.67
FT-MIR	3020 – 2950cm^{-1}	*Tej*	100.59 – 102.44	*
	3020 – 2950cm^{-1}	*Tella*	99.43 – 102.83	
UV-NIR	1660 – 1720 nm	*Areke*	99.08 – 104.96	99.41 – 100.94

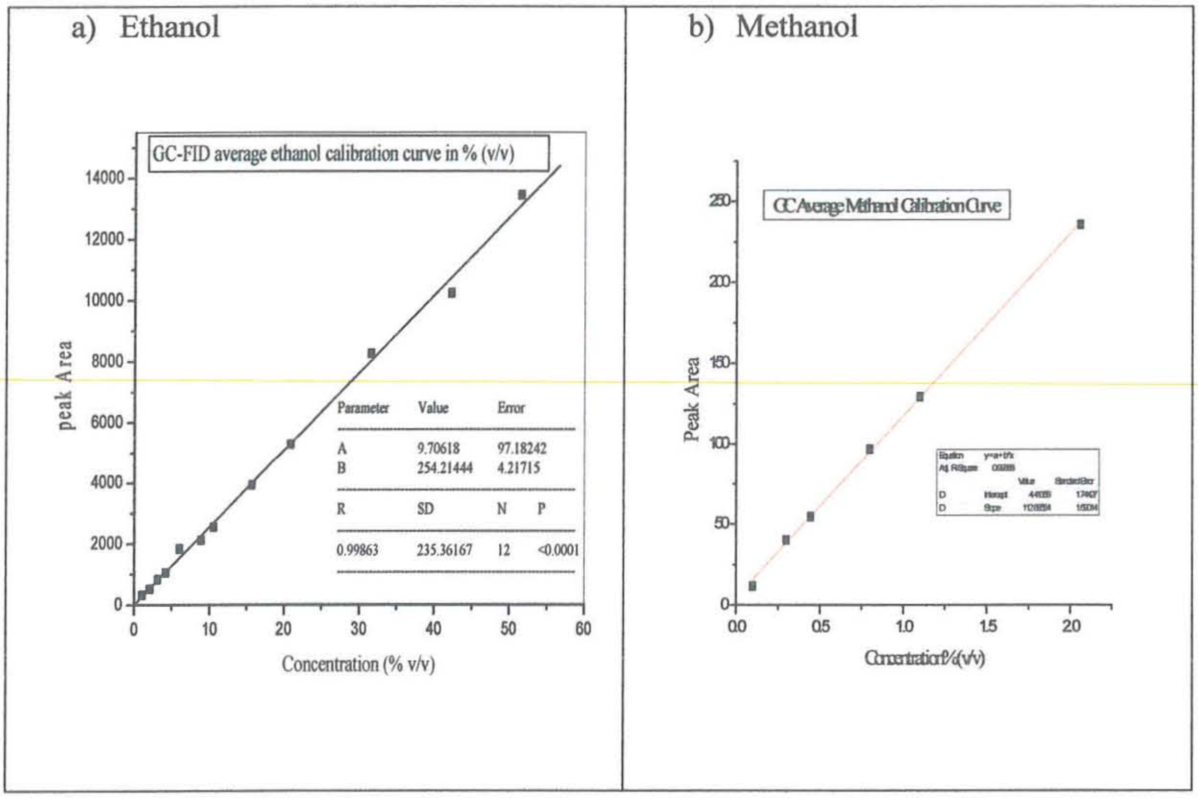
**Not available*

Based on data obtained the matrix effect of the samples is not considerable. Therefore, the proposed techniques of FT-MIR in the region 1180 – 950 cm^{-1} and UV-NIR in the region 1660 – 1720 nm were appropriate to quantify alcohol contents in distilled alcoholic beverages. In addition, FT-MIR method in the region 3020 – 2950 cm^{-1} was suitable to determine ethanol content without any sample preparation except filtrations.

4.5 Comparison of the developed methods with GC-FID

Comparison was conducted to check the accuracy the proposed FT-MIR and UV-NIR methods in relation to the reference method, GC-FID. For the comparison of the developed methods with the reference method the chromatographic condition was repeatedly by measuring different replicates of ethanol and methanol standard solutions prepared in % (*w/w*). Then, the peak area, retention time and peak symmetry of ethanol and methanol were compared. As a result, the following chromatographic conditions were obtained: 0.5 μL injection volume, 80 °C oven temperature, 210 °C injection port temperature, 0.3 bar pressure, 0.7 mL/min flow rate, 300 °C detector temperature and ECTm-5 capillary column.

Accordingly, a series of standard solution of ethanol and methanol were prepared and analyzed in duplicate. The calibration of ethanol and methanol were based on peak area versus concentration in % (*w/w*) were plotted. Then, the obtained average calibration equations were converted to % (*v/v*) with equations of density versus % (*w/w*) y= -0.0014 x + 0.9975 for ethanol and y= -0.00183x + 0.99818 for methanol. The calibration curves of ethanol and methanol are presented in Figure 27.



a) Ethanol

b) Methanol

Figure 27: GC-FID calibration curve in % (*v/v*), (a) ethanol and (b) methanol.

As indicated in Fig. 27, the calibration equation of ethanol and methanol in % (*v/v*) has coefficient determination of 0.9973 and 0.999, respectively, with p value < 0.0001.

Comparison of the developed methods with the reference were performed to check the accuracy of the proposed FT-MIR and UV-NIR techniques in relation to the reference

GC-FID method using commercial samples. In addition to the commercial samples the analyses of traditional alcoholic beverages were performed to make certain the performance of the developed methods.

Thus, the proposed FT-MIR and UV-NIR methods were applied for the direct determination of alcohol contents in traditional and commercial distilled alcoholic beverages, comparing the analysis with GC-FID. The results obtained were statistically analyzed with one-way ANOVA using Origin Lab\Origin 8 at 95% confidence level (Table 4 and Figure 28).

Table 4: Comparison of UV- NIR, FT-MIR and GC-FID for ethanol determination in some alcoholic beverages

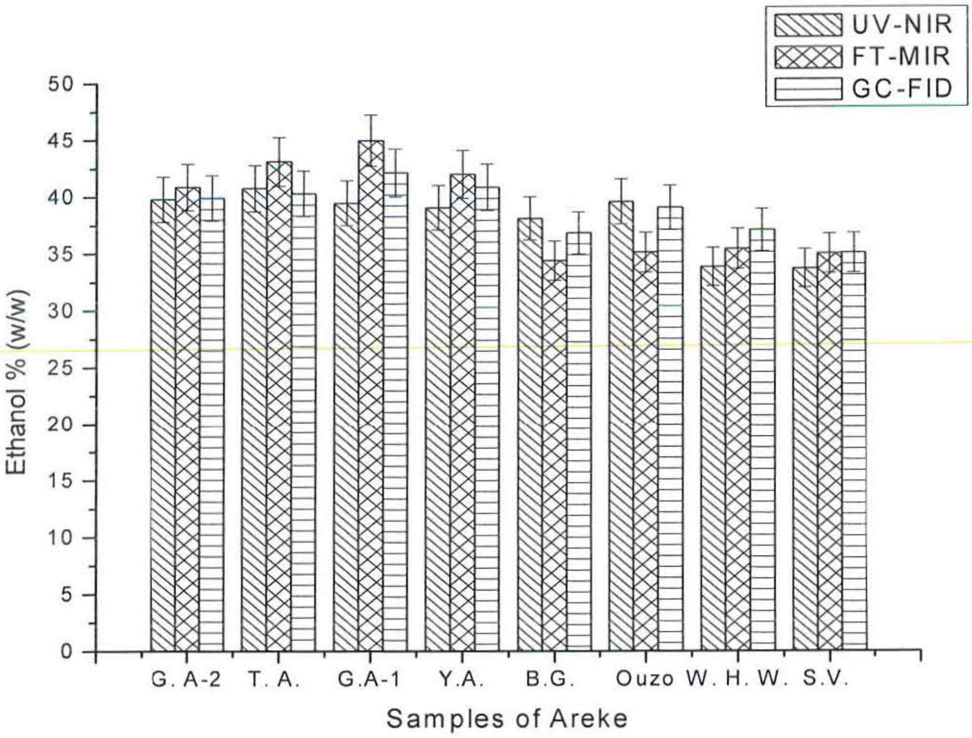
S.No	**Sample**	**Labeled amount % (*v/v*)**	**UV-NIR**		**FT-MIR**		**GC-FID**	
			% (*v/v*)	**% L. a.**	**% (*v/v*)**	**% L. a.**	**% (*v/v*)**	**% L. a.**
1	Yegebto *Areke* - 1	*	42.6 ± 0.09	-	41.5 ± 0.16	-	42.1 ± 1.43	-
2	Terra- *Areke*	*	43.6 ± 0.07	-	43.8 ± 0.73	-	42.5 ± 0.67	-
3	Yegebto *Areke* - 2	*	42.2 ± 0.10	-	43.1 ± 1.07	-	44.5 ± 1.95	-
4	Yekoso *Areke*	*	41.8 ± 0.05	-	42.6 ± 0.15	-	43.1 ± 1.43	-
5	Baro Gin	41	40.7 ± 0.04	100.7	39.8 ± 0.25	97.1	38.8 ± 2.91	94.6
6	Ouzo	41	42.3 ± 0.01	96.9	41.4 ± 0.15	101.0	41.2 ± 1.29	100.5
7	White Horse Whisky	40	36.0 ± 0.09	90.0	37.1 ± 0.30	92.8	39.0 ± 0.74	95.1
8	Stolichnaya Vodka	37	36.0 ± 0.09	97.3	35.4 ± 0.73	95.7	36.8 ± 0.73	99.5

* Local alcoholic beverages which do not have labeling

$$\% \text{ Labeled amount} = \frac{Amount\ found}{Labelled\ amount} \times 100$$

The three techniques compared do not have any significant difference in the result obtained.

Therefore, FT-MIR can be used for the qualitative analysis to distinguish the presence of methanol. For the distilled alcoholic beverages in the absence of methanol UV-NIR can compensate the limitation of FT-MIR. As a result, direct analysis of samples without dilution can be done using UV-NIR.



G.A.-1 and G.A.-2 (Yegibto 1 and 2), Y.A. (Yekosso), Terra (T.A), Baro Gin (B.G), White Horse whisky (W.H), Stolichaniya Vodka (S.V.)

Figure 28: Comparison of UV-NIR, FT-MIR and GC-FID on measuring the amount of ethanol in distilled alcoholic beverages.

4.6 Real Samples Analyses Results and Discussion

4.6.1 Distilled alcoholic beverages

The level of ethanol, pH and titratable acidity in *Areke* samples collected from various part of the country is illustrated in Table 5. The samples were collected from areas which are major producing and source of varieties.

Table 5: pH, titratable and % (*v/v*) of ethanol in *Areke* samples collected from different parts of Ethiopia

Ser. No	**Sample sites**	**Types of *Areke* sample**	**pH ± SD**	**Titratable acidity ± SD (g/100ml)**	**Amount of ethanol in % (*v/v*)**	
					FT-MIR	**UV-NIR**
1.	Arisi Negele	Yekoso 1	6.97 ± 0.01	0.72 ± 0.02	36.2 ± 0.8	37.8 ± 0.1
2.	Arisi Negele	Yekoso 2	6.99 ± 0.02	0.57 ± 0.01	36.7 ± 0.6	37.7 ± 0.1
3.	Arisi Negele	Yekoso 3	6.97 ± 0.01	0.66 ± 0.02	36.3± 0.5	37.7 ± 0.1
4.	Arisi Negele	Cereal 1	6.35 ± 0.01	0.39 ± 0.02	43.9 ± 0.4	44.2 ± 0.1
5.	Arisi Negele	Cereal 2	6.78 ± 0.01	0.33 ± 0.02	42.6 ± 0.2	44.2 ± 0.1
6.	Butajira	Yekoso 1	5.89 ± 0.03	0.22 ± 0.03	30.3 ± 0.7	31.6 ± 0.1
7.	Butajira	Yekoso 2	6.48 ± 0.01	0.36 ± 0.02	34.9 ± 0.7	36.0 ± 0.1
8.	Butajira	Cereal 1	6.74 ± 0.02	0.33 ± 0.01	39.4 ± 0.2	40.4 ± 0.1
9.	Butajira	Cereal 2	6.46 ± 0.01	0.31 ± 0.01	33.1 ± 0.6	34.4 ± 0.7
10.	Dembecha	Dagim 1	4.13 ± 0.01	0.77 ± 0.02	45.7 ± 0.3	46.8 ± 0.1
11.	Dembecha	Dagim 2	4.14 ± 0.02	0.72 ± 0.01	46.1 ± 0.1	46.7 ± 0.1
12.	Dembecha	Dagim 3	4.09 ± 0.01	0.79 ± 0.01	45.6 ± 0.1	46.7 ± 0.1
13.	Dembecha	Dagim 4	4.10 ± 0.01	0.69 ± 0.01	45.8 ± 0.1	46.7 ± 0.1
14.	Dembecha	Yegibto 1	4.50 ± 0.01	0.69 ± 0.02	43.4 ± 0.2	44.6 ± 0.1
15.	Dembecha	Yegibto 2	4.52 ± 0.02	0.72 ± 0.01	43.7 ± 0.1	44.5 ± 0.1

4.6 Real Samples Analyses Results and Discussion

4.6.1 Distilled alcoholic beverages

The level of ethanol, pH and titratable acidity in *Areke* samples collected from various part of the country is illustrated in Table 5. The samples were collected from areas which are major producing and source of varieties.

Table 5: pH, titratable and % (*v/v*) of ethanol in *Areke* samples collected from different parts of Ethiopia

Ser. No	Sample sites	Types of *Areke* sample	pH ± SD	Titratable acidity ± SD (g/100ml)	Amount of ethanol in % (*v/v*)	
					FT-MIR	UV-NIR
1.	Arisi Negele	Yekoso 1	6.97 ± 0.01	0.72 ± 0.02	36.2 ± 0.8	37.8 ± 0.1
2.	Arisi Negele	Yekoso 2	6.99 ± 0.02	0.57 ± 0.01	36.7 ± 0.6	37.7 ± 0.1
3.	Arisi Negele	Yekoso 3	6.97 ± 0.01	0.66 ± 0.02	36.3± 0.5	37.7 ± 0.1
4.	Arisi Negele	Cereal 1	6.35 ± 0.01	0.39 ± 0.02	43.9 ± 0.4	44.2 ± 0.1
5.	Arisi Negele	Cereal 2	6.78 ± 0.01	0.33 ± 0.02	42.6 ± 0.2	44.2 ± 0.1
6.	Butajira	Yekoso 1	5.89 ± 0.03	0.22 ± 0.03	30.3 ± 0.7	31.6 ± 0.1
7.	Butajira	Yekoso 2	6.48 ± 0.01	0.36 ± 0.02	34.9 ± 0.7	36.0 ± 0.1
8.	Butajira	Cereal 1	6.74 ± 0.02	0.33 ± 0.01	39.4 ± 0.2	40.4 ± 0.1
9.	Butajira	Cereal 2	6.46 ± 0.01	0.31 ± 0.01	33.1 ± 0.6	34.4 ± 0.7
10.	Dembecha	Dagim 1	4.13 ± 0.01	0.77 ± 0.02	45.7 ± 0.3	46.8 ± 0.1
11.	Dembecha	Dagim 2	4.14 ± 0.02	0.72 ± 0.01	46.1 ± 0.1	46.7 ± 0.1
12.	Dembecha	Dagim 3	4.09 ± 0.01	0.79 ± 0.01	45.6 ± 0.1	46.7 ± 0.1
13.	Dembecha	Dagim 4	4.10 ± 0.01	0.69 ± 0.01	45.8 ± 0.1	46.7 ± 0.1
14.	Dembecha	Yegibto 1	4.50 ± 0.01	0.69 ± 0.02	43.4 ± 0.2	44.6 ± 0.1
15.	Dembecha	Yegibto 2	4.52 ± 0.02	0.72 ± 0.01	43.7 ± 0.1	44.5 ± 0.1

16.	Dembecha	Yegibto 3	4.48 ± 0.01	0.75 ± 0.01	43.9 ± 0.1	44.6 ± 0.1
17.	Dembecha	Yegibto 4	4.49 ± 0.03	0.66 ± 0.01	43.6 ± 0.1	44.6 ± 0.1
18.	Dembecha	Yemar 1	4.74 ± 0.01	0.69 ± 0.01	41.8 ± 0.1	42.0 ± 0.1
19.	Dembecha	Yemar 2	4.76 ± 0.01	0.72 ± 0.01	40.4 ± 0.3	41.9 ± 0.1
20.	Debrebirhan	Yekosso (white) 1	4.50 ± 0.01	0.21 ± 0.01	44.2 ± 0.4	45.5 ± 0.2
21.	Debrebirhan	Yekosso(white) 2	4.50 ± 0.01	0.36 ± 0.01	43.5 ± 0.6	44.0 ± 0.3
22.	Debrebirhan	Yekosso (brown) 1	5.3 ± 0.01	0.33 ± 0.01	37.1 ± 0.7	39.2 ± 0.1
23.	Debrebirhan	Yekosso (brown) 2	6.00 ± 0.01	0.62 ± 0.02	41.0 ± 0.5	43.4 ± 0.1
24.	Debrebirhan	Dagim Yekosso (white)	4.60 ± 0.01	0.39 ± 0.02	49.3 ± 0.5	51.4 ± 0.1
25.	Debrebirhan	Cereal	4.50 ± 0.01	0.33 ± 0.01	41.8 ± 0.1	43.6 ± 0.1
26.	Debrebirhan	Yemar	4.37 ± 0.06	0.72 ± 0.03	35.7 ± 0.6	37.7 ± 0.1
27.	Debrebirhan	Yenech shinkurt 1	5.30 ± 0.01	0.46 ± 0.03	35.3 ± 0.5	37.7 ± 0.1
28.	Addis Ababa	Cereal 1	5.43 ± 0.01	0.41 ± 0.01	43.8 ± 0.7	43.6 ± 0.7
29.	Addis Ababa	Yekosso	5.94 ± 0.03	0.42 ± 0.01	42.6 ± 0.2	41.8 ± 0.1
30.	Addis Ababa	Yegibto 1	6.27± 0.01	0.42 ± 0.03	41.5 ± 0.2	42.6 ± 0.1
31.	Addis Ababa	Yegibto 2	5.74 ± 0.01	0.39 ± 0.02	43.1 ± 0.2	42.2 ± 0.1

The pH values with in the same type (same ingredients) but varied in the sample sites showed a significant difference ($P < 0.05$). In this study, the pH values of *Areke* samples varied between 4.10 to 6.99 (Table 5).

There was also a significant difference in pH values among: Yekoso Areke, samples collected from Arsi-Negele, Addis Ababa, Butagera and Debrebrhan; Yegibto Areke collected from Addis Ababa and Dembecha and Cereal (Terra) *Areke* collected from Addis Ababa, Arsi-Negele Butagera and Debrebrhan. The mean pH-value of Yekoso

Yegibto and Cereal *Arekes* in this study was 5.95, 6.33 and 5.0, respectvely. The mean pH value of Dagim and Yemar *Areke* collected from Dembecha and Debrebrhan was 4.11 and 4.62, respectively.

Considering the pH and Titratable acidity values, in this study Dagim, Yegibto and Yemar *Areke* samples may generally be considered as acidic.

The proposed FT-MIR and UV-NIR methods based on C-O and O-H stretching, respectively, were applied for the determination of alcohol levels in traditional alcoholic beverages, *Arekes*. The ethanol content of the samples ranges from 31.6–51.4% (*v/v*).

The results obtained indicated that there is no significant difference between the two techniques. Though, the two methods were comparable.

In this study the alcoholic contents of Yekosso (30.3–44.2), Yemar (35.7–41.8), Yegibto (41.5–43.9), Dagim (45.6–49.3) and Cereal *Arekes* (33.1–43.9) are with the mean values of 38.3, 39.3, 43.2, 46.5 and 40.5% (*v/v*) respectively. Accordingly, Dagim *Arekes* have higher alcoholic contents than the rest since they are double distilled. However, the single distilled types commonly called ordinary (in Amharic *Terra*) such as Yekosso, Yemar, Yegibto and Cereal *Arekes* have comparable level of alcoholic contents irrespective of the additional ingredients they have.

For Terra and Dagim *Arekes* the mean values obtained are 40.5 and 46.5% (*v/v*) whereas the reports according to Debebe, 2006, are 37.2 and 48.0% (*v/v*), respectively. The obtained results are different from the reported ones. On the other hand, when the alcoholic content of *Areke* is compared with other distilled beverages, it is in the range of liqueurs' alcoholic content, 20-50% (*v/v*) (Wang et al., 2003).

According to (Fite & Tadesse) the methanol content of *Areke* was 320.87 ppm. However, in this study no methanol in the samples of *Areke,* were detected (Figure 29). In addition, the recovery test of methanol obtained in this study showed both methods have ≥ 99 % (*v/v*). This indirectly confirms the absence of methanol in the samples.

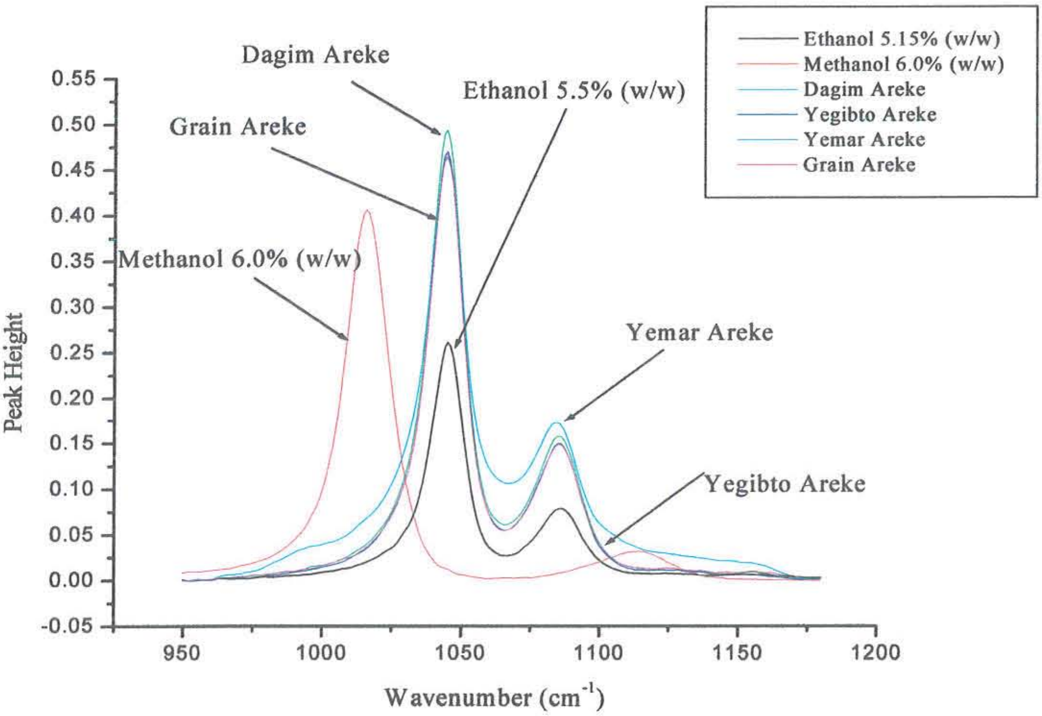


Figure 29: FT-MIR spectra of different *Areke* samples compared with pure ethanol and methanol solutions

The absence of methanol was observed and this might be due to the fact that methanol contents in the samples were lower than the instrument detection limits or the sample does not contain methanol, at all.

4.6.2 Fermented alcoholic beverages *Tella* and *Tej*

The level of ethanol, pH and titratable acidity in *Tella* and *Tej* samples collected from various part of Addis Ababa are illustrated in Table 6 and 7. The samples were collected from the 10 sub- cities of Addis Ababa. The ethanol content was determined by the proposed FT-MIR method on the basis of C-H stretching in the region 3020 – 2950 cm^{-1}.

Table 6: pH, titratable acidity as acetic acid (g/100 mL) and ethanol% (v/v) in *Tella* samples collected from all sub- cities of Addis Ababa, Ethiopia

Sub - cities	**pH ± SD**	**Titratable acidity as acetic acid (g/100 mL) ± SD**	**Ethanol content using FT-MIR**	
			% (*v/v*) range	**Mean% (*v/v*) ± SD**
Addis Ketema	3.69 ± 0.02	3.17 ± 0.001	3.01 - 4.16	3.59 ± 0.03
Akaki Kality	3.69 ± 0.02	2.91 ± 0.002	3.37 - 4.93	4.15 ± 0.05
Arada	3.77 ± 0.01	3.18 ± 0.001	3.32 - 4.13	3.73 ± 0.03
Bole	3.77 ± 0.01	2.65 ± 0.002	2.22 - 3.37	2.80 ± 0.06
Gulele	3.70 ± 0.01	3.21 ± 0.002	3.24 - 3.57	3.41 ± 0.08
Kirkos	3.70± 0.01	2.64 ± 0.001	3.04 - 5.07	4.06 ± 0.04
Kolfe Keraneo	3.57 ± 0.01	3.39 ± 0.003	4.04 - 4.14	4.09 ± 0.04
Lideta	3.62 ± 0.01	3.30 ± 0.002	2.32 - 5.65	3.99 ± 0.03
Nefas Silk Lafto	3.66 ± 0.01	3.05 ± 0.003	2.04 - 2.11	2.08 ± 0.04
Yeka	3.74 ± 0.01	2.78 ± 0.001	1.94 - 2.31	2.13 ± 0.12

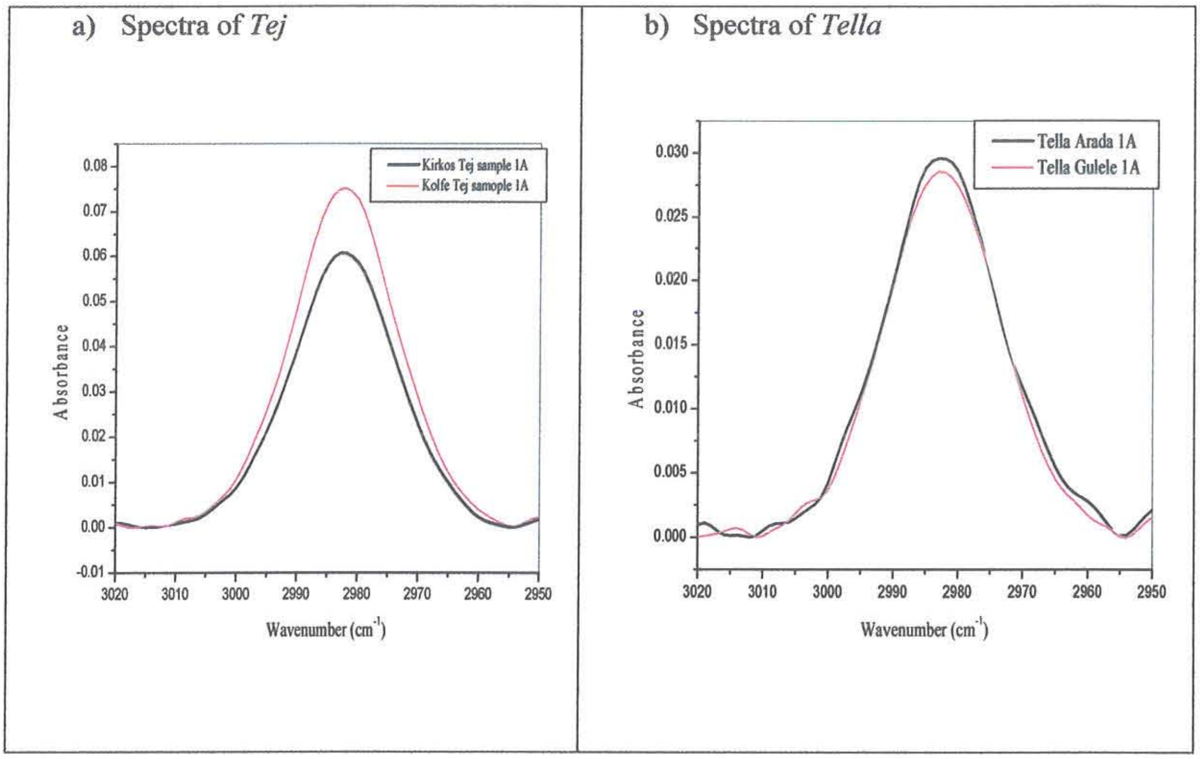
Table 7: pH, titratable acidity as acetic acid (g/100 mL) and ethanol % (*v/v*) in *Tej* samples collected from all sub- cities of Addis Ababa, Ethiopia

Sub - cities	**pH ± SD**	**Titratable acidity as acetic acid (g/100 mL)**	**Ethanol content using FT-MIR**	
			% (*v/v*) range	**Mean% (*v/v*) ± SD**
Addis Ketma	3.60 ± 0.01	2.74 ± 0.001	6.79 - 9.15	8.11 ± 0.14
Akaki Kality	3.71 ± 0.01	2.50 ± 0.006	7.09 - 7.42	7.26 ± 0.09
Arada	3.91 ± 0.01	2.19 ± 0.004	8.10 - 9.15	8.63 ± 0.21
Bole	3.61 ± 0.02	2.85 ± 0.004	7.03 - 7.85	7.44 ± 0.05
Gulele	3.64 ± 0.01	2.82 ± 0.006	6.80 - 8.93	7.87 ± 0.13
kirkos	3.47 ± 0.01	3.33 ± 0.005	7.03 - 7.30	7.17 ± 0.11
Kolfe Keraneo	3.58 ± 0.01	3.01 ± 0.009	8.93 - 9.36	9.15 ± 0.04
Lideta	3.58 ± 0.01	3.74 ± 0.003	8.1 - 9.15	8.63 ± 0.24
Nefas silk Lafto	3.70 ± 0.02	2.36 ± 0.003	7.01 - 7.31	7.16 ± 0.10
Yeka	3.63 ± 0.02	2.63 ± 0.004	6.8 - 8.94	7.87 ± 0.14

The pH- values of *Tella* samples in this study varied between 3.57 – 3.77 with mean value of 3.69. Similarly, the pH- values of *Tej* samples in this study varied between 3.47–3.91 with mean value of 3.64. Titratable acidity of *Tella* samples varied from 2.64 to 3.39 g/l00 mL with mean value of 3.03. The titratable acidity of *Tej* samples were varied from 2.19 to 3.74 g/l00 mL with mean value of 2.82.

The mean pH value of Ethiopian traditional alcoholic beverages, *Tella* and *Tej*, varies from report to report Sahle and Gashe, (1991), in his study of *Tella* fermentation, reported that the pH values range from 4.5 – 4.8. Similar studies by Debebe (2006) reported that the pH values of *Tella* and *Tej* varies between 3.87 – 5.03 and 3.74 – 3.79, repectively.

The ethanol content of *Tella* and *Tej* were analyzed in the region 3020-2950cm^{-1}. The C-H stretching absorbance spectra of the samples were done by tangential base line fit using Microcal Origin lab 6. Such spectra of *Tella* and *Tej* samples were presented in Figure 30.


a) Spectra of *Tej*

b) Spectra of *Tella*

Figure 30: FT-MIR spectra of ethanol on the basis of C-H stretching a) *Tej* and b) *Tella*.

As indicated in Fig 30, the maximum absorbance of ethanol in the region 3020 to 2950 cm^{-1} was at 2983 cm^{-1} for both *Tella* and *Tej* samples. The absorbances were as a result of C-H stretching of ethanol. In this region there were no significant effect caused by the C-H stretching of glucose, fructose and sucrose.

The ethanol content of *Tella* varied between 1.94 – 5.65% (*v/v*). The mean value of ethanol in this study was 3.40% (*v/v*). The maximum ethanol content was found in Nefas-Silk Lafto and the minimum in Akaki sub-cities (Table 6 and Figure 31).

Likewise, the ethanol content of *Tej* samples collected from Addis Ababa varied between 6.79 – 9.36% (v/v) with mean value 7.91% (v/v). In this study from 10 sub-cities two samples of Tej from each sub-cities were analyzed and the mean values and the ranges for each sub-city were reported in Table 7. The minimum and maximum ethanol content of *Tej* samples found in this study was from Nefas- Silk Lafto and Kolfe Keraneo sub-cities, respectively (Figure 31).

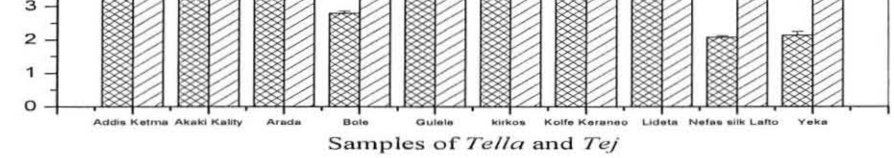


Figure 31: Comparison of ethanol content of *Tella* and *Tej* samples with respect to sample sites.

According to (Sanni, 1993), honey wine of Eastern Europe had an alcoholic content of 6.4 to 16.6% (*v/v*). Meads had 6.6 to 14.2% (*v/v*) (Steinkraus, 1983). Honey wines of the US market were reported to have an alcoholic content of 12.2 to 20.8% (*v/v*) (Steinkraus, 1983; Vogel & Gobezie, 1983) and reported that if fermentation of *Tej* goes to completion, the final alcoholic content would be 7 to 13% (*v/v*).

According to the report of (Bahiru et al., 2001) the total alcohol content of *Tej* samples varied between values as low as 2.7% (*v/v*) to values as high as 21.7% (*v/v*). Similar study reported by Debebe, (2006) the mean ethanol content of *Tej* samples varied between 8.91 to 13.71% (*v/v*) with mean value of 11.47% (*v/v*).

In comparison to the above beverages, the mean ethanol content of *Tej* is above that of the report of (Fite & Tadesse) and in the range of that of Bahiru et al (2001), (Sanni, 1993) and Steinkraus (1983) reports but, it is below US honey wines (Steinkraus, 1983).

The levels of alcohol in Ethiopian traditional alcoholic beverages were determined by FT-MIR spectroscopy techniques. Compared to densitometer and pychnometeric methods there were no sample preparations. Because, these methods require distillations, the methods are not selective for ethanol only; other alcohols such as methanol and higher alcohols are distilled in the distillation process and determined simultaneously. As a result it is a time consuming method and the results are not accurate. However, in this study the levels of ethanol were determined by selective, sensitive, and accurate technique of FT-MIR spectroscopy and the result obtained were very reliable. In samples of *Tella* and *Tej* methanol was not detected. This might be due to lower methanol content of the samples than the instrument detection limits or absence of methanol in the samples, at all.

4.7 Health impact assessment of *Areke*

The level of alcohol in *Areke* obtained in this study varies between 35 – 51 % (*v/v*). The home-made Areke showed a higher variation in their alcoholic strengths. It must be noted that the preferred alcoholic beverage in Ethiopia is *Areke* due to many reasons such as medicinal values. Therefore, *Areke* is usually diluted with pure ethanol in order to increase the strength of *Areke.*

In this health impact assessment of Ethiopian traditional alcoholic beverage, *Areke* in major producer and consumer areas of Ethiopia questionnaires and interviews were used. Structured type questionnaires (close-ended) were used and filled by producers and consumers (Appendices A and B) with the following considerations (ingredients used, how to distill the alcohol, customer type, age, drinking frequency and amount and problems associated with health such as eye problem, liver, etc. A total of 150 questionnaires for producers and consumers were used in this study.

The result obtained in this assessment indicates that the variances of alcoholic strength in *Areke* depended mostly on the preferences of producers who usually make it for personal use and may add other ingredients like flowers of *Kosso* (*Hagenia Abyssinica*), Honey, *Tenaadam* and *Nechshinkurt* (*Ruta Chalepensis* and *Allium Satiuum*) and *Gibto* (*Lupinus Albu or White Lupin*) in order to change the taste, the strength and to increase the medicinal values beside with the major components (Wheat, Barely, Maize, Teff, Dagussa (*Elusine coracann*), *Gesho* leaves (*Rhamnus prenoids*) and Water).

In the distillation process the first distillate were not discarded by the producers. As a result the concentration of ethanol and methanol were high.

According to WHO (2011) heavy episodic drinking is the measure alcohol consumption risks. In this report, it is defined as drinking at least 60 grams or more of pure alcohol on at least one occasion in the past seven days. However, the result obtained in the assessment indicates that 87% of the interview consumed 700 mL of *Areke* per day.

This health impact assessment of Areke showed that out of 150 people interviewed 86.67% were heavy drinkers (82.67% of men and 4% women). Out of 82.67% men 23% were older age groups greater than 50 years.

Compared to the older age groups men in the 18 – 35 are groups are more likely to be abstinent than women in the same age group. Most drinking occasions took place in small bars the evenings. However, in Butajira, Arsi- Negele and Debrebrhan people drink from morning to mid-night.

Heavy alcohol drinkers in this study had health problems than moderate drinkers and also had some kind of social problem in past years including problems with family and work. Being a frequent drinker was strongly associated with quarreling and experiencing physical aggression. According to this survey 17.4% of 150 people had eye problems (older men) and high rate of liver cirrhosis problems.

According to WHO, (2009) about sixty disease categories have been identified in which alcohol is a contributing factor. Alcohol contributes to or is the sole cause of chronic and acute health problems because of its direct toxic effects on organs (as in alcohol liver

Cirrhosis), its intoxicating properties (as in accidents and injuries) and because it is a dependence producing substance.

In this study methanol content were not detected. This indicates that the eye and liver problems is not due to methanol.

Since uncontrolled alcoholic beverages like Areke are cheap and easily accessed by the consumers, the risk of alcohol related problem is serious in Addis Ababa, Butajira, Arsi-Negele and Debreberhan.

5. Conclusions and Recommendations

5.1 Conclusions

The developed and validated technique, FT-MIR and UV-NIR allows the direct determination of ethanol and methanol in distilled alcoholic beverages. In the presence of methanol FT-MIR in the region 1180-950cm^{-1} can qualitatively detect and quantitatively determine the ethanol content of distilled alcoholic beverage, *Areke*. Therefore, FT-MIR technique in the 1180-950cm^{-1} range is suitable for the direct determinations of ethanol and methanol in distilled alcoholic beverages and spirits (*Areke*).

In the absence of methanol as it was observed in this study UV-NIR can quantify the level of ethanol without any dilution. Therefore, the proposed techniques can work complementarily.

The proposed FT-MIR technique in the region 3020-2950cm^{-1} based on C-H stretching absorption band is suitable for fermented alcoholic beverages '*Tella* and *Tej*' ethanol content determinations without any sample pretreatments except filtrations.

The ethanol content of Ethiopian traditional alcoholic beverages *Areke, Tej* and *Tella* varies considerably, *Areke* between 31.6 – 51.4% (*v/v*); 6.79 - 9.36% (*v/v*) for *Tej* and 1.94 -5.65% (*v/v*) for *Tella.* In this study methanol was not detected.

Compared with previously reported procedures involving IR measurements, the recommended techniques provides excellent analytical quality which are comparable or better than those offered by other published methods including the on-line vapor phase generation FTIR (Pérez-Ponce & de La Guardia, 1998).

Generally, the proposed techniques, FT-MIR in the region between 1180-950cm^{-1} and UV-NIR in the range 1720-1660 nm for distilled alcoholic beverages and FT-MIR in the region between 3020-2950cm^{-1} for fermented alcoholic beverages is in excellent agreement with the results obtained from Gas Chromatographic measurements. In addition, the techniques are rapid, low cost and reliable for the direct determination of ethanol and methanol in common Ethiopian alcoholic beverages (*Areke, Tej and Tella*)

5.2 Recommendations

The densititometric and pychnometric methods have been used for a long period of time as the approved reference method to determine the alcoholic strength in different alcoholic beverages. Since, this measurement typically has to be preceded by a distillation step which is time-consuming and also require special training of personnel to obtain valid and reproducible results, it is less reliable.

Since, the results in this study showed that the developed methods can be successfully applied to control or regulate the level of alcohol in common Ethiopian traditional alcoholic beverages with in a short period. Therefore, it is recommended to use these methods by conformity assessment bodies in Ethiopia and students who are working on alcoholic beverages and alcohols.

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

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7. Appendices

Appendix- A

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES
Center of Food science and nutrition

Questionnaire for Ethiopian alcoholic beverage, *Areke*

To be filled by producers

Researcher: Abel Anberbir

Advisor: Dr. Mesfine Redi

Professor Negussie Retta

Research Topic: Level of alcohols in Ethiopian common beverages using spectroscopic techniques

Dear Respondents:

I would like to express my sincere appreciation and deepest thanks in advance for your generous time and frank and prompt responses.

Objective

The purpose of this questionnaire is to find out the reality of alcohol consumption, the customer type, type of ingredient used and the health impact due to alcoholic beverage of Areki in the major production and consumption areas of Deberaberhan, Addis Ababa, Dembecha, Butagera and Arsi-Negele. For the researcher as partial fulfillment of academic requirements of MSc. degree in food science and nutrition; it will predicts the impact of Areki on health together with the result obtained from experimental analysis and finally for other prospective researchers as a stepping stone to carry out further investigation.

General Guideline:

Please put a tick " √ " mark for those questions that are followed by choices and write your short and precise answers for those followed by blank spaces .As an important input of this study your frank response is greatly appreciated. Your valuable supports in responding to these questions have paramount importance to the success of the study.

The survey is anonymous, so please answer as truthfully and accurately as possible. Hence, I ask you in all regard to fill the questionnaire carefully. The quality and quantity of information you provide determines the ultimate reliability of the study.

Confidentiality

I want to assure you that this research is only for academic purpose authorized by then Addis Ababa University, Center of food science and nutrition program coordination office and the result will not be presented for other purposes. Thus, your ideas and comments are highly honored and kept confidential. To create conducive environment for your free and genuine responses, you are not required to write your name.

Contact Address

Name: Abel Anberbir

Mobile: 09-11-78-98-53 e-mail:abelanberbir@yahoo.com

Thank you again!!!!

Code ☐ **(For the researcher use only)**

1. Gender

   ☐ Male ☐ female

2. In which age group are you?

   ☐ Less than 20 ☐ 21-30 ☐ 31-40

   ☐ 41-50 ☐ 51-60 ☐ 61 and above

3. What is your highest and recent educational status?

   ☐ No education ☐ below grade 4

   ☐ 12 grades complete ☐ Certificate

   ☐ College diploma ☐ First degree and above

4. Type of ingredient used to make Areki?

   ☐ Barely ☐ Sorgum ☐ water

   ☐ Wheat ☐ maize ☐ Gesho

   ☐ Degussa ☐ teff ☐ others

5. If your answer is other please specify the type of ingredients used?

   ☐

6. How long you are in producing Areki?

   ☐ Less than 1 year ☐ 1 – 2 years

   ☐ 2 -5 years ☐ 5 – 10 years

   ☐ Greater than 10 years

7. How many customers use your beverage (Areki) per day?

☐ Less than 10 ☐ 10- 20 customer ☐ 20 – 30 customer

☐ 30 -50 customer ☐ 50 – 100 custome☐ Greater than 100 customer

8. Have you ever remove the first distillate of Areki?

☐ Yes ☐ No

9. If your answer is yes for question number 8, do you know the reason why the first distillate of Areki is removed?

☐ Yes ☐ No

10. If you know the reason why the first distillate of Areki is removed please specify?

☐

Thank you for your participation!!!

Appendix- B

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Center of Food science and nutrition

Questionnaire for Ethiopian alcoholic beverage, *Areke*

To be filled by consumers

Researcher: Abel Anberbir

Advisor: Dr. Mesfine Redi

Professor Negussie Retta

Research Topic: Level of alcohols in Ethiopian common beverages using spectroscopic techniques

Dear Respondents:

I would like to express my sincere appreciation and deepest thanks in advance for your generous time and frank and prompt responses.

Objective

The purpose of this questionnaire is to find out the reality of alcohol consumption, the customer type, type of ingredient used and the health impact due to alcoholic beverage of Areki in the major production and consumption areas of Deberaberhan, Addis Ababa, Dembecha, Butagera and Arsi-Negele. For the researcher as partial fulfillment of academic requirements of MSc. degree in food science and nutrition; it will predicts the impact of Areki on health together with the result obtained from experimental analysis and finally for other prospective researchers as a stepping stone to carry out further investigation.

General Guideline:

Please put a tick " √ " mark for those questions that are followed by choices and write your short and precise answers for those followed by blank spaces .As an important input of this study your frank response is greatly appreciated. Your valuable supports in responding to these questions have paramount importance to the success of the study.
The survey is anonymous, so please answer as truthfully and accurately as possible. Hence, I ask you in all regard to fill the questionnaire carefully. The quality and quantity of information you provide determines the ultimate reliability of the study.

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I want to assure you that this research is only for academic purpose authorized by then Addis baba University, Cnter of food science and nutrition program coordination office and the result will not be presented for other purposes. Thus, your ideas and comments are highly honored and kept confidential. To create conducive environment for your free and genuine responses, you are not required to write your name.

Contact Address

Name: Abel Anberbir

Mobile: 09-11-78-98-53 e-mail:abelanberbir@yahoo.com

Thank you again!!!!

Code ☐ **(For the researcher use only)**

1. Have you ever consumed alcohol?

   ☐ Yes ☐ No

2. At what age did you start consuming alcohol?

   ☐

3. Why did you start consuming alcohol?

   ☐ Curiosity ☐ Because you 'felt like it' (Bored)

   ☐ Influence of an adult ☐ Peer pressure

4. Which type of common alcoholic beverage is your favorite?

   ☐ tella ☐ tej

   ☐ Areki ☐ others

5. If your choice is Areki which type is your choice?

   ☐ Yekosso Areki ☐ dagem Areki

   ☐ Yegebto Areki ☐ terra Areki

6. How often do you drink?

   ☐ 3-5 times a week ☐ once a week ☐ only on weekends

   ☐ On special occasions ☐ every day

7. Do you engage in binge drinking? (5 or more drinks in a sitting).

   ☐ Yes ☐ No

8. In the last two weeks, how many times have you had 5 or more drinks at a sitting?

   ☐ 1 – 3 ☐ 3 – 5 ☐ 5 -7 ☐ more than 7 times

9. Have you ever passed out or experienced memory loss due to drinking?

   ☐ Yes ☐ No

10. Do you drink to escape pain, either physical or emotional?

☐ Yes ☐ No

11. In which type of beverage you fill pain, either physical or emotional?

☐ Yekosso Areki ☐ dagem Areki

☐ Yegebto Areki ☐ terra Areki

12. Have you ever been told you have liver trouble? Cirrhosis?

☐ Yes ☐ No

13. Have you ever been told you have eye trouble?

☐ Yes ☐ No

14. Have you ever had delirium tremens (DT's) (state of mental illness usually found in long-term alcoholics who attempt to give up alcohol consumption), severe shaking, heard voices, or seen things that were not there after heavy drinking?

☐ Yes ☐ No

Thank you for your participation!!!