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Addis Ababa University

ROASTED BARLEY ADDITION TO COFFEE: DETECTION OF THE ADULTRANT BARLEY MARKER USING GAS CHROMATOGRAPHY, SPECTROSCOPY AND TRAINED PANELISTS

By

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DECLARATION

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

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List of Abbreviations

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CSA	Central Statistics Agency
EI	Electron Ionization
GC-MS	Gas chromatography/Mass spectrophotometer
GDP	Gross Domestic Product
IR	Infrared
ISO	International Organization for Standardization
LSD	Least Significant Difference
NIR	Near infrared
NIST	National Institute of Standards & Technology
PPM	Part Per Million
SE	Standard Error
SPME	Solid Phase Microextraction
SPSS	Statistical Package for Social Science
UV/VIS	Ultra Violet/ Visible

Abstract

The objectives of this research were to detect the adulterant roasted barley at different concentrations (5 to 20 % w/w) in roasted & ground coffee using GC, UV spectroscopy, Infrared (IR) spectroscopy, trained panelists and also total carbohydrate determination by difference. The samples used were pure barley, pure coffee, medium & dark roasted coffee and barley mixtures (50:50%w/w). Compounds identified in GC were 3-Eicosene,(E),2-Tetradecene,(E)-, 2,4 Di tert butyl phenol and E-15-Heptadecenal based on comparison of retention times with National Institute of Standards and Technology library. Infrared spectroscopy qualitative transmission measurement of pure coffee & medium roasted coffee admixture (5 to 20% w/w barley) showed that the infra red spectra among 5 % to 20 % w/w barley addition to coffee were not resolved though it was possible to identify the peak regions useful to discriminate the roasted barley from roasted coffee IR spectra using caffeine molecule, lipid content and starch presence .The addition of barley,10% to 20% to Harar coffee showed significant ($p<0.05$) increase in total carbohydrate content, however the crude ash, crude fat and crude protein content decreased significantly ($p<0.05$).The UV spectroscopy analysis showed that it can discriminate pure coffee from adulterated coffee, 5% to 20% w/w barley based on UV absorbance measurement. Mean while, trained panelists Quantitative Descriptive Analysis (QDA) sensory evaluation indicated that, there was no significant ($p\geq0.05$) difference among the adulterated coffee 5% to 20% w/ w barley using pure coffee as a reference with respect to aroma, acidity, bitterness and body sensory notes. Moreover, p^H measurement revealed no significant ($p\geq0.05$) difference between pure Harar coffee and adulterated coffee brews, 5% to 20 % w/ w barley. In conclusion, coffee adulteration with roasted barley would have a potential to be detected by gas chromatography analysis and it was observed that Eicosene,(E)-,2-tetradecene,(E)-,2,4 Di tert butyl phenol and E-15-Heptadecenal were tentatively identified to be further used for detecting the adulterant barley in roasted and ground coffee. Infrared spectroscopy couldn't resolve the addition of 5 % to 20 % w/ w roasted and ground barley to pure coffee. UV spectroscopy analysis resulted in discriminating pure roasted and ground Harar coffee from the adulterated coffee as low as 5% w/ w barley addition. Meanwhile, Sensory evaluation resulted in not discriminating the adulterated coffee samples 5%, 10% and 20% w/ w barley despite pure coffee was presented as a reference. The total carbohydrate determination by difference could be useful in verifying the adulterant barley in roasted & ground coffee.

Key words: Adulterant barley, Caffeine, GC, Harar coffee, IR spectroscopy, Panelists, p^H, QDA, UV spectroscopy

1. Introduction

1.1. Background

Arabica Coffee originated from Ethiopia and discovered by a goat herder named kaldhi around 850 A.D who noticed his goats getting frisky after eating the coffee berries of the plant. Coffee before 700 years was used as food, wine and also as a medicine which then became a popular beverage (Nolan, 2001).Then it spread to Yemen, Arabia and Egypt (Wang and Ho, 2009).

Coffee is the second most consumed drink next to water (Butt and Sultan, 2011), the most traded in the world market after petroleum and about 10 billion US dollar is traded in world market per annum (Cordella et.al, 2002). Meanwhile, about 500 billion cups of coffee are consumed per year by about one third of the population of the world (Butt and Sultan, 2011) and about 1.5 billion cups of coffee are consumed every day worldwide (Nolan, 2001).

Arabica coffee covers 75% of coffee production in the world (George et al, 2008).The Ethiopian Arabica coffee assured its economic importance in the world market (Oestreich-Janzen, 2010). Robusta coffee covers one third of the production. Coffee is produced by 70 countries; Brazil 35%, Vietnam 15%, Colombia 10% and Indonesia 5% of the world coffee production. The rest of the market share is occupied by Ethiopia, Mexico and India (Oestreich-Janzen, 2010). Ethiopia stood 5th in the world coffee production (Oeistrich-Janzen, 2010) and seventh largest exporter in the world, the largest coffee producer and exporter in Africa besides 15 million Ethiopians are dependent on it for their livelihood (Petit, 2007). Arabica coffee has more market share and higher price than Robusta coffee, the longer, expensive Arabica beans are more preferred to the small, round Robusta beans which produce thicker brew (Higdon and Frei, 2006).

Coffee is a complex chemical mixture consisting of about a thousand chemicals such as carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids, and phenolic compounds (Higdon and Frei, 2006). Arabica coffee caffeine content ranges from 0.6 to 1.9% mean of 1.2% however Robusta coffee has mean caffeine content of 2.2% (Belay, 2011).

Coffee along with its ceremony is a sign of showing hospitality and also serves as a day to day psycho stimulant for many through its enjoyable roast aroma though it is mainly used for its hedonistic and physiological effect (Dorea and da Costa, 2005). Coffee popularity has its essence in caffeine presence. caffeine intake of 20-200 mg/day results in alertness, concentration, increased motivation, energy boost and efficiency on work (Olmos et al, 2009).The physiological effects of caffeine are stimulation of central nervous system, acute increment of blood pressure, increasing metabolic rate and diuresis (Higdon and Frei, 2006).

In the Ethiopian culture, there is a well preserved traditional coffee ceremony with long and strong coffee drinking consuming more than 40 percent of produced coffee for domestic consumption (Petit, 2007). Ethiopia produced 3,768,231.72 quintals of Coffee in 2011/12 with a yield of 7.3 (CSA, 2011/12) whereas Barley production in Ethiopia in 2011/12 is 15,852,869.21 quintals with a yield of 16.72 (CSA, 2011/12).

Fraudulent profit seeking from food mispresentation is a well known fact since long time ago (Dennis, 1998). There are three types of adulteration approaches in commercial roast & ground coffee ; addition of cheap materials like barley, brown sugar, caramel, coffee husks& straw, maize and soya bean, the second is mixing of two coffee varieties, cheaper Robusta coffee with pure Arabica coffee and the other form of coffee adulteration is mixing of coffee beans growing at one region with cheap coffee beans from another region (Sano et al, 2002).

Adulteration detection is a technical problem since detecting adulterants which have similar physical characteristics to roast and ground coffee once processed requires the best analytical method(s) (Reis et al., 2013)

1.2. Statement of the Problem

Arabica coffee is a high value commercial product and produced in many tones around the world, it can be vulnerable to adulteration by mixing it with cereals, coffee twigs, Robusta coffee, brown sugar (Jham et al., 2007). The other reason for its adulteration could be its physical characteristics resemblance while roasted and ground to cereals, seeds, roots and parchments (Fontes et al., 2001; Varvolgyi et al., 2013). Moreover, the increase in coffee prices paves the way for adulteration to take place (Briandet et.al., 1996).

Economic adulteration of food poses ill effect on consumers by diminishing the nature, quality, originality, nutritional value (Dennis, 1998). Moreover, it creates unfair competition, destabilizes the market and disrupts the regional economy and even affects the national economy (Cordella et.al, 2002).

Most of the analytical methods for adulteration detection are time demanding, expensive and laborious which made them less suitable for routine analysis however there is an urge by food chemists, regulatory bodies and quality personnel for fast, more powerful, cheaper and cleaner analytical method for adulteration detection usable for routine analysis (Alpdogan et al, 2002).

Ethiopia is the largest Arabica coffee bean producer and coffee consumer in Africa, thus economic adulteration of roast and ground coffee is most likely to prevail. According to Shewayrga and Sopade, dark roasted Barley coffee drink is prepared from barely in very limited situations when there is shortage of proper coffee beans in North Eastern Ethiopia (Shewayrga and Sopade, 2011).

Thus adulteration detection is essential for food manufacturers, regulatory bodies and retailers due to the possible negative economic and health effect on consumers (Sano et al., 2002). The adulteration detection of roast ground coffee needs sound analytical methods for ensuring authenticity and eliminating trading fraud (Casal et al., 2000).

Coffee consumers should not be provided with adulterated coffee beverages and/or adulterated roast ground coffee. Moreover, pure roast ground coffee and/or coffee beverage retailers should not be affected through unfair competition (Dennis, 1998).

Barley is described to be coffee adulterant in literatures and Varvolgi et.al. (2013) compared electronic tongue, near IR and untrained sensory panelists techniques to detect barley in Robusta coffee meanwhile Briandet et al. (1996) used FT-IR to detect glucose, starch or chicory in instant coffee, Sano et.al (2003) described adulteration detection of coffee husk & straw, maize, brown sugar and soy bean addition to coffee by digital imaging, where as Reis et.al (2013) studied detection of barley, corn, coffee husks and spent coffee grounds by using Fourier Transform Infrared (FT-IR). Nogueira and do Lago (2009) used capillary zone electrophoresis to detect cereals and coffee husks. Thermal lens and p^H measurement analysis to detect corn grain in coffee ,Fourier transform infrared spectroscopy with chemo metrics of adulteration detection of instant coffee ,use of high performance liquid chromatography for detection of corn addition in coffee using tocopherol marker are worth to mention (Nogueira and do Logo, 2009), solvent extraction followed by HPLC analysis for detection of coffee adulteration by barley, adulterations detection by a trained sensory panel using volatile profile of roasted barley in coffee and SPME GC-MS analysis of the headspace volatiles of roasted barley in coffee (Oliveira et.al., 2009).

Adulterant addition to coffee is mispresentation of coffee which disguise consumers to purchase inferior quality with higher price, results in unfair market competition to the loyal traders who sell authentic product moreover it erodes consumer confidence on roast and ground coffee which eventually lead to less market place (Dennis, 1998) to the roast and ground coffee unless roast and ground coffee quality control programs are initiated urgently.

Coffee roaster companies in Ethiopia are emerging nowadays and value added coffee export is of crucial importance to the country however, coffee quality certification is not applied on roast and ground coffee for domestic consumption to detect adulterants unlike the green coffee beans quality inspection. Thus, in this study different analytical methods and/or the marker compounds for detecting the adulterant barley in coffee were identified.

1.3. Objectives

1.3.1. General Objective

- To identify the marker of the adulterant barley using GC, spectroscopy and trained panelists.

1.3.2. Specific Objectives

- To identify volatile compounds in roast & ground pure barley, pure coffee and coffee barley mixtures (50:50% w/w; medium and dark roast) using GC.
- To discriminate the adulterant barley in coffee (5% to 20% w/w) using UV and infrared spectroscopy.
- To verify the addition of barley (5% to 20% w/w) in roast and ground coffee by trained panelists.
- To determine the total carbohydrate composition of the pure coffee and adulterated coffee, roast & ground (5% to 20% w/w).

2. Review of Literature

2.1. Coffee

The coffee plant belongs to Rubiaceae family , there are three species of coffee for commercial production, *Coffea arabica*, has 44 chromosomes thus cannot form cross hybrid, grow in humid, evergreen tropical forests, require moderate temperature, 950-1950 m, 4 to 6 m tall perennial tree where as *Coffea robusta*, 22 chromosomes , resists diseseases, lower requirement of humidity, temperature and altitude, 8 to 12 m tall (Oestreich-Janzen, 2010) and *Coffea liberica* , the latter being severely affected by infection in 1940 by_*Fusarium xylaroides* causative agent of tracheomycosis which consequently made Arabica and Robusta coffee to be the two commercially important species (Ramalakshmi and Raghavan, 1999).

The caffeine content of Arabica laurina variety bean 0.62%, typica 1.05%, Mundo novo 1.11% and catuai, 1.34%. The most important component of coffee is caffeine, a white crystalline, odorless substance that plays a great role in coffee’s poularity.Caffeine,1,3,7 trimethyl xanthine ,is a purine alkaloid found naturally in leaves ,seeds or fruits of 63 plant species (Belay, 2011), a secondary metabolite produced for defense against herbivores, mollusks, insects, fungi or bacteria (Oestrech-Janzen, 2010). From 100 ml cup with a brew strength of 55 g/l of roast ground coffee in water with 60% Arabica and 40% Robusta provides an average 100 mg caffeine meanwhile the brew strength of coffee brew can be 40 g/l as to Dutch nutritional table or 55 g/l German common use, mean between 40 g/l and 70 g/l and the ISO 6668:2008 coffee brew strength of 70 g/l of water (Oestreich-janzen, 2010).

Coffee consumption is not intended for nourishment nevertheless magnesium 63.7 microgram/cup of 100 ml can be supplied to the body (Dorea and da Costa, 2005).

Coffee beans contain carbohydrates, fats, water, protein, vitamins, organic acids, alkaloids such as caffeine, minerals and flavoring substances Polyphenols (primarily chlorogenic acids) are other important constituents of coffee having anti inflammatory, anti bacterial and anti atherosclerotic effects (Sadowska- Rockie et al., 2015).

Caffeine is quickly absorbed from the stomach, small intestine and distributed to all tissues within 15 minutes to 1 hour after ingestion with maximum plasma level and caffeine is excreted out of the body with half life of 3 to 6 hour (Olmos et al, 2009).

Beside to coffee, cocoa beans, cola nuts and tea leaves are the most common sources of caffeine. Caffeine intake survey studies reveal as coffee is 70% caffeine source where as soft drinks are 16% and tea 12% (Butt and Sultan, 2011).

Coffee in moderate amounts, 3-4 cups of coffee per day is reported to benefit health by preventing type 2 diabetes mellitus, Parkinson's disease and reduce the suicide risk, prevention of certain type of cancer. However, studies show that excessive coffee consumption is reported as risk for cardiovascular diseases, certain type of cancer, osteoporosis and hip fracture, mineral deficiencies (Higdon and Frei, 2006). Excessive consumption of coffee is known as caffeinism, 600 mg/day caffeine intake results in anxiety, irritability and insomnia (Monica et al, 1999).

The Arabica coffee beans suffer from leaf rust disease and drought though the Ethiopian Arabica coffee is known to resist leaf rust diseases related to the fact that Ethiopia is the center of Arabica coffee diversity. Studies by Brazilians discovered that Ethiopia also possesses three naturally decaffeinated varieties of coffee having caffeine content of 0.07%. Ethiopia holds 6% of the world's coffee ex situ collection, Ethiopian coffee genetic materials are represented in world collections and the country is the only region where Arabica coffee is found as wild forest species (Worako et al., 2011).

The ethiopian traditional coffee ceremony is well established and unique in the world which involves roasting and grinding the coffee beans, brewing the coffee in clay pot and consuming it three times as 'abol','tona' and 'bereka'. The coffee consumers not only enjoy the coffee drink but also the psycho stimulation from the coffee aroma while roasted. The traditional coffee ceremony has been generating job for women since the coffee consumers prefer it. Mean while, in the globalized world people tend to prefer processed foods or those foods with less preparation time, coffee is no exception, however the high price of coffee to other cereals makes it a target of adulteration, for instance 1 kilo gram coffee bean price is about tenfold than barley significantly low cost crop which is known to resist drought and adverse climates.

2.2. Coffee Adulterants

The deliberate misrepresentation of food ingredients on product package labels is a potential problem in the food industry. Consequently, food forensics is an emerging discipline which ensures the authenticity of food products such as coffee. (Casal et al., 2000; Cordella et al., 2002 ; Reis et al, 2013; Dennis, 1998).

Adulteration is a serious problem because consumers are supplied with products of non compliance to specifications and regulatory standards which is a trade fraud leading to unfair competition, which disrupts the stabilized local and global economies there by loyal traders are surpassed by frauder's reputable competition (Oliveirra et al, 2009).

Coffee can be adulterated to gain inappropriate profit while ignoring consumer's right to enjoy the aroma, flavor and body of pure coffee. Green coffee beans on international trade can be visually identified as Arabica or Robusta however once roast and ground it is difficult whether cheap Robusta is added to an expensive Arabica or coffee from one region is mixed with another region or coffee substitutes are added to coffee by their physical characteristics (Nougeira and do Lago, 2009).

Arabica coffee is most favored for its improved organoleptic features and thus it is sold at higher prices meanwhile, the price is related to several factors such as origin and coffee species, Adulteration with intent to deceive is usually carried out by admixture of cheaper products and materials which are usually difficult to detect by consumers and by simple routine analytical techniques, with high-priced commodities being usually the main target for adulteration (Oliveirra et.al, 2009). Adulteration occurs frequently due to analogy to physical characteristics by cereal materials like corn & barley (Varvolgi et al, 2013).

Since coffee resembles the physical characteristics (particle size, texture and color) to that of roast and ground cereals, seeds, and parchements and so on (Casal et. al, 2000; Oliveirra et.al, 2009), it is difficult to detect adulterants by visual inspection (Ebrahimi-Najafabadi et al., 2012).

Some of the coffee adulterants are barley, chicory, fig, brown sugar, caramel, coffee straw and husks, and soy bean. (Casal et al. , 2000; Ebrahimi-Najafabadi et al., 2012 ;Oliveirra et al. , 2009 ;Sano et al., 2003; Jham et al, 2007; Reis et al., 2013; Nogueira and do Lago, 2009). The adulterants are of lower price and have similar characteristics while roast and ground to roast and ground coffee.

In Brazil for instance as being the world's largest producer and the second largest consumer and it is stated as coffee adulteration is prevalent hence there are studies how to detect adulterants in coffee powders (Oliveira et al, 2009). In Brazil, coffee husks are recognized as non intentional thus are considered as impurities however the addition of brown sugar, caramel, maize and soy bean is considered as intentional, meanwhile, the adulterants that are more frequently used are husks, sticks, roasted corn, cocoa, seed husks ,barley, wheat middling, brown sugar, soy bean and others (Sano et.al, 2003) .

Adulteration of coffee can be economical, to increase the bulk and reduce the coffee powder so as to get fraudulent profit which could not be possible to recognize by physical characteristics .Coffee adulterants are many in number as described earlier thus a specific method is necessary to resolve adulteration problem. Corn is described as the most widely used adulterant of Arabica coffee due to its significantly lower cost, Jham et al. (2007) used gamma tocopherol amount of corn as marker of adulteration in Arabica coffee .

2.3. Detection of Coffee Adulteration

Detection of adulteration of food and food products was reported as early as the 18th century (Olieverra et.al, 2009). The earliest adulteration detection was carried out by Arthur Hill Hassal (Dennis, 1998) in 1861 by using microscope to prove authenticity of coffee products which he sorted 31 as being adulterated with chicory, roasted wheat and burnt sugar out of 34 samples examined, which reveals that inappropriate profit gain from adulteration, physical contamination of food by mixing low quality materials to a superior quality food product has been practiced more than a century ago (Denis, 1998).

Economic adulteration of a food product has been prevalent in the world (Briandet et al., 1996; Reis et al, 2013; Prodolliet and Hischenhuber, 1998). Assessment of instant coffees for their authenticity by the UK Ministry of Agriculture, Fisheries and Food in 1995 using the sugar content showed that 15% of the samples examined are not pure (Dennis, 1998; Briandet et al., 1996).

Adulteration of coffee by extending or substituting a food by cheaper materials like barley is a fraudulent practice which affects coffee consumers by diminishing the nature, quality ,originality ,nutritional value and creates unfair market competition , destabilizes the market and affects the regional economy may even pose impact on the national economy of a country thus adulteration detection is of great importance for coffee consumers, roaster companies and regulatory bodies and for retailers (Briandet et al., 1996; Sano et al., 2003; Cordella et al, 2002).

Assurance for quality of roasted coffees has attracted widespread attention for controlling and preventing coffee adulteration, also given the great difference in the final sale price (Ebrahimi-Najafabadi et al. 2012). For instance, in Brazil within one year of coffee quality certification enhanced internal coffee consumption by 2.1% in 2008 (Moares and Bolina, 2010).

Detection of adulteration with impurities or mixtures in roasted and ground coffee is essential to reveal the presence of extraneous materials, to determine whether the adulteration is carried out intentionally or non intentionally. However, adulteration detection is referred as a technical problem since detecting multiple adulterants which have similar physical characteristics to coffee after roast and ground requires the best analytical method(s) (Reis et al., 2013).

The escalating number of studies on the development of analytical tools for the detection and quantification of adulterations of food and food products in published works in recent years has shown that the trade fraud has become a common practice in all food sectors all around the world (Olieverra et.al, 2009).

Thus, efficient methods are extremely important for controlling authenticity of roast and ground coffee to assess trade fraud, to address the increased need and concern of consumers about their food quality which often need the development and use of new analytical techniques (Cordella et al., 2002; Murray et al., 2002; Sano et al., 2003; Prodolliet and Hischenhuber, 1998). Consequently different analytical methods are devised to detect adulterants for ensuring authenticity of a food product, food authenticity methods include spectroscopy (ultraviolet, near infrared, mid infrared , visible and Raman spectroscopy), isotopic analysis, chromatography, electronic nose, polymerase chain reaction, enzyme linked immunosorbent assay, thermal analysis and chemometrics (Jham et al, 2007).

Mean while, the different analytical methods used are classified as physical, chemical, biochemical, and microscopic techniques; Thermal lens and p^H measurement analysis to detect corn grain in coffee ,Fourier transform infrared spectroscopy with chemo metrics of adulteration detection of instant coffee, use of high performance liquid chromatography for the detection of corn addition in coffee using tocopherol marker are worth to mention (Nogueira and do Logo, 2009), solvent extraction followed by HPLC analysis for the detection of coffee adulteration by barley, adulterations detection by a trained sensory panel using volatile profile of roasted barley in coffee and SPME GC-MS analysis of the headspace volatiles of roasted barley in coffee (Oliveira et al., 2009). Varvolgi et al. (2013) described electronic tongue, near infrared analysis and untrained panelists sensory analysis are some techniques for detection of roasted barley in coffee (Varvolgi et al., 2013).

However, a methodology based on solvent extraction followed by HPLC analysis for detection of coffee adulteration by barley and coffee husks showed that coffee husks can be satisfactorily be detected in coffee nevertheless chromatographic profiles of the extracts obtained for coffee and barley were quite similar, with the exception of the absence of caffeine in the chromatograms obtained for barley, which revealed that the method was not effective for detection of adulteration of coffee by barley (Oliveira et.al., 2009).

However, in many cases, most of the techniques for the detection of adulteration are expensive, complex and/or time consuming. For this reason, a fast, reliable, low-cost technique with ease of implementation for routine analysis represents a very attractive alternative for adulteration and varietal identification purposes (Ebrahimi-Najafabadi et al., 2012).

Detecting adulterants in a food by using one analytical technique could not be enough to detect the different multiple adulterants usually encountered thus it is vital to combine analytical methods in assessing authenticity of a food product. These analytical techniques must address an important number of problems providing information about processing, quality control, ensuring compliance with food and trade laws, adulteration, contamination, product tampering, and the chemical composition of foods (Denis, 1998).

Most analytical methods studied for detecting adulterants, despite the methods effectiveness are time consuming, subjective and many times of conflicting results which made them less suitable for routine analysis however adulteration detection based on physical measurements only are gaining much interest than using chemical tracers such methods include photo acoustic-spectroscopy, IR spectroscopy, scanning electron microscopy and thermal lens spectrometry but most analytical methods for soluble coffee are based on carbohydrate content. Consequently, there is a constant concern by food chemists, regulatory bodies and quality personnel for fast, more powerful, cheaper and cleaner analytical method for adulteration detection applicable for routine analysis (Alpdogan et al, 2002).

2.3.1. Gas Chromatography (GC) Analysis

GC is one of the most universal separation techniques used in food analysis, mainly in volatile composition studies, food applications of GC include the detection of lipids, volatile compounds (alcohols, esters, ketones, aldehydes), additives, pesticides, and compounds responsible for aroma and flavor. Moreover, GC is also used to detect product adulterations (Ibanez and Cifuentes, 2001).

Couplings such as GC-MS or GC-FTIR are well established in food science, and they are considered as mature and routine techniques. They combine the separation power of GC with the unambiguous identification capability and structural information provided by the coupled spectroscopic technique, the use of GC-MS is ubiquitous in food analysis.

The direct coupling of GC with MS (mainly using EI based interfaces) has been widely applied for characterization and quantification of volatile, lipids, flavor compounds, contaminants, etc. for a large number of food matrices. Also, GC-MS has been abundantly used for the characterization of products and in food authentication (Ibanez and Cifuentes, 2001).

The main limitation of this technique is that it cannot be applied to nonvolatile, highly polar, and/or thermolabile compounds (Ibanez and Cifuentes, 2001). Gas chromatography is used for separating volatile organic compounds. Numerous detectors for this kind of chromatography have been developed since the 1950s, and several of them have high sensitivity and/or selectivity (Cordella et al., 2002).

Roasting is usually carried out in the temperature range of 120–230 °C, this allows for the development of aroma, color and flavor, which are essential for the characterization of natural and cereal coffee. However, at the same time, roasting may lead to the formation of dangerous compounds such as furan, acrylamide and polycyclic aromatic hydrocarbons (Sadowska - Rockie et al., 2015).

A total number of 26 volatile compounds were identified and it was confirmed that the aldehydes, alcohols and ketones and furan were the predominant constituents of volatile compounds in barley, 1 octene -3-ol, 3- methylbutanal, 2- methyl butanal, hexanal, 2-hexenal, 2-heptenal, 2-nonenal and decanal are the key odorants in barley (Cramer et al., 2005). Depending on the different stages of formation of the volatile compounds that appear at different malting stage, they can be divided into groups, group I, represents those that only exist in the barley itself and vanish in the subsequent malting and roasting process; group II, those compounds that exist in barley and the malting process, but which disappear after serious roasting and group III are those formed in the malting process but which vanish in roasting treatment (Dong et al, 2013).

The classical approach to flavor analysis involves isolation of volatile compounds from matrix followed by pre concentration, separation and identification techniques (Dong et.al, 2013). Simple solvent extraction, supercritical fluid extraction, solid phase micro extraction are used for extracting volatile compounds (Dong et al, 2013).

Simple solvent extraction followed by concentration under nitrogen or in rotary evaporator is one of the earliest method used to recover flavor compounds in foods (Zhou et al., 1999).

Bianchi et al. (2005) compared SPME GC-MS and comprehensive GC x GC-Time Of Flight (TOF)-MS for qualitative characterization of volatile compounds in roasted barely. Oliveira et al. (2009) detected adulteration of ground roasted coffee with roasted barely using SPME-GC-MS.

2.3.2. Infrared (IR) Spectroscopy Analysis

IR mostly used in chemical, pharmaceutical and food industries (Ribeiro et. al., 2011). Infra red techniques can have future prospects in assuring food authenticity and quality assessment (Elena and Alejaandro, 2001). One of the spectroscopic techniques most commonly used in the food product adulteration area or quality control of manufactured products is infrared spectroscopy, the major advantage of IR spectroscopy rather than other previously used techniques lies in its nondestructive nature, spectroscopic techniques yield structural information of a molecular fingerprint (Cordella et al, 2002).

Over the last decades, the application of near infrared spectroscopy (NIRS) as a fast and non-destructive technique for the authentication of food samples has become widespread thanks to the advances in chemometrics. Furthermore, it allows to directly analyze solid samples without any complex physical/chemical pre-treatment. Thus, several studies concerning NIR applications in food quality and authentication assessment have been reported (Ebrahimi-Najafabadi et al., 2012)

IR need less detection time, little or no sample preparation and it is of good adoptability, the near infrared spectra can be recorded in reflectance, transmission and interactance mode, by the vibration of C-H, O-H and N-H which are useful to produce complex chemical and physical information (Qu et al., 2014). IR spectroscopy is a well acknowledged method to prove authenticity of a product and in quality control (Cordella et al., 2002; Varvolgi et al., 2013).

The NIR spectroscopic analysis of food adulteration is a recent focus of many studies as it provides fast result with minimal preparation, on invasiveness and its easiness to use. NIRS has been used to discriminate Arabica and Robusta coffee varieties in pure or blended samples, to determine caffeine content, trigonelline and chlorogenic acid, total sugar, minerals, roasting degree of beans, also is used to determine quality of espresso coffee (Ribeiro et al, 2011).

As to other techniques , NIR spectroscopy is useful for adulteration detection ,by using the tracers for detection such as proteins, carbohydrates, fatty acids, pigments and organic acid and thereby authentication of a food product (Prodoliet and Hischenhuber, 1998).

The broad band at about 3400 cm^{-1} corresponds to many vibration of OH groups, also minor effect of NH functional groups. The two sharp peaks at 2925 cm^{-1} and 2855 cm^{-1} are attributed to methyl and methylene presence, asymmetric and symmetric stretching of C-H bonds in aliphatic chain. These peaks are reported to indicate caffeine presence however these peaks are found in coffee waste residue studied thus the two peaks are likely attributed to the presence of larger amount of lipids in coffee (Pujol et al., 2013). Peaks in 2250-1850 cm^{-1} absorbance peaks are more intense in the spectra for roasted barley than in the spectra of coffee, thus these spectra are important in discriminating pure coffee from adulterated ones as starch is present in barley and not present in coffee (Reis et al., 2013).

The sharp peak at 1742cm^{-1} is attributed to the lipids, carbonyl vibration in aliphatic esters or in triglycerides. The low intensity bands at 1665 cm^{-1} and 1523 cm^{-1} are attributed to lipids and fatty acid C =C vibrations and C= C vibrations of aromatic rings from lignin moieties (Pujol et al., 2013)

The bands at 1461cm^{-1} is from C-H bending of CH_3 groups. The bands at 1061, 1113, 1163, 1242, 1376 cm^{-1} are attributed to chlorogenic acid. the vibrations from 900-1400cm^{-1} are attributed to characteristics of carbohydrates showing several vibrations of C-H, C- O -C, C-N and P-O, FT-IR cannot differentate peaks between chlorogenic acid and polysaccharides (Pujol et al., 2013).

2.3.3. UV Spectroscopy Analysis

UV-VIS spectroscopy analysis is an easier, cheaper and more suited for routine laboratory analysis of roasted coffee adulteration detection in comparison to gas chromatography/mass spectrophotometer, high performance liquid chromatography, fourier transform infra red, mid infra red, near infra red and digital image processing (Souto et al., 2015). UV-VIS spectrophotometer is cheap and available in many laboratories (Atomssa & Gholap, 2011). Oestreich-Janzen described chromatographic and spectroscopy methods as the standard analytical determination methods for caffeine analysis (Oesterich-Janzen, 2010).

Caffeine is the most studied and analyzed compound in coffee and is useful to discriminate green coffee varieties. Moreover, is also used to authenticate coffee using spectroscopy technique (Casal et al, 2000). Thus it is important to access adulteration detection methods which are faster, reproducible and of low cost using equipments accessible in many laboratories for routine analysis.

2.3.4. Sensory Analysis

In psychometrics, the role of sensory evaluation is of great importance as it helps to determine food quality. Descriptive sensory analysis is regarded as the most comprehensive and vital to analyze sensory characteristics of a product (Seo et al., 2009).

Coffee quality can be assessed pre harvest and post harvest; among the pre harvest coffee quality factors include the species type, coffee variety, area of cultivation, seed maturation, effect of microbes and fertilization. Post harvest coffee quality inspection includes storage, enzyme and microbial fermentation of the coffee beans and coffee cup quality, generally factors affecting coffee quality can be at production system, the aspect & composition of green or roasted coffee and coffee brew (Ribeiro et al., 2011).

Descriptive sensory analysis techniques are one of the well developed methods in the carrier of sensory professionals, because the qualitative aspect of a food will be quantified and described by sensory panel (Murray et al., 2001). To get thorough information about sensory attribute of a food the role of descriptive sensory analysis is undeniable (Seo et al., 2009). It involves detection and description of sensory attributes and also is important in forming relationship between descriptive sensory test and instrumental analysis techniques (Murray et al., 2001).

Coffee quality can be defined in terms of organoleptic cup quality, physical appearances and inherent chemical constituents such as sugars, caffeine, volatile and non volatile phenolic contents of a green bean consequently coffee quality is considerably affected by many factors such as inappropriate post harvest practices which are major problems of Ethiopian coffee, since numerous factors are affecting coffee quality thereby post harvest processing techniques largely contribute to the decline in coffee quality (Sualeh et al., 2014). Beverage quality assessment is done using organoleptic characteristics by trained coffee tasters, this method is sufficiently reliable for use as a basis of selection in quality improvement programs (Kathurima, 2009).

Well-trained and experienced expert coffee tasters are called “cuppers.”They are responsible for the assessment of many cups of coffee per day for years; they are trained to distinguish slight differences among coffee samples and to describe a wide range of coffee. They use a more specific and precise language to describe the sensory quality of coffee (Hayakawa et al., 2010)

In the case of roasted coffee, adulterations can usually be perceived by a trained sensory panel, thus, indicating that an analysis of the volatiles profile, which is expected to be singular for a particular adulterant, could provide a reliable method to verify adulteration of ground roasted coffee (Oliveira et al., 2009).

Among the commonly used contaminants, barley is particularly difficult to detect especially at low concentrations, and even methodologies that are based on the chemical profile are not effective for this specific contaminant (Oliveira et.al.2009). Coffee quality has tremendous role in the coffee industry, it refers to a coffee product that has desirable, clean, raw and roasted appearance, superb flavor and clean cup taste, having balanced acidity and body (Sualeh and Mekonnen, 2013).

The flavor is combination of retro-nasal aroma impression and taste, well-balanced acidity interacting with a fruity and roasty note, rounded by a slight, coffee-like bitterness, is often considered to be a good coffee flavor whereas the aroma is perceived ortho-nasally and measured via analysis of the headspace of the coffee brew Meanwhile, the body, also called mouth- feeling or texture, is related to the total solids and sometimes it is also linked to fat or fatty acid content. Coffee can also be classified as to type of drink, where sensory tests are conducted (cup testing) to assess a number of parameters, such as aroma, acidity, bitterness, taste, astringency, and so forth, so that at the end of the test it is classified into one of the following classes: Soft, pleasant aroma, and sweet taste; Hard, bitter taste; Rioysh, slightly chemical taste with typical iodoform flavor; Rio, Inferior flavor with excessive chemical taste. Arabica beans have good acid balance and chocolaty to flowery aroma while Robusta coffee beans more bitter taste and woody to earthy aroma (Sualeh et. al. ,2014), a reliable sensory panel is a prerequisite to quality data generated from sensory evaluation (Kathurima, 2009).

Sensory evaluation is inevitable to determine organoleptic characteristics of coffee quality measurement which involves trained assessors for discriminating pure coffee from adulterated ones (Sualeh and Mekonnen, 2013). The link between coffee price and coffee quality made coffee susceptible for adulteration which requires monitoring the composition of coffee, adulteration detection and quality assessment of coffee (Santos et al., 2012).

2.3.5. Total Carbohydrate Content Analysis

Carbohydrates in coffee are useful as aroma binders in coffee beans, for keeping the viscosity and thickness of coffee beverage, foam stability of espresso coffee beverage and polymeric carbohydrates are beneficial as dietary fiber and in prebiotics (Arya and Rao, 2007). This effects of carbohydrates on organoleptic characteristics of coffee led to their addition as non coffee carbohydrates as roasted and ground chicory roots and starchy cereal grains which increases intentional adulteration of commercially available coffee powders (Arya and Rao, 2007).

Carbohydrate composition of adulterated coffee can reveal information whether the coffee is pure or adulterated (Nogueira and Do logo, 2009). Carbohydrate composition determination of products are gaining increasing significance as they not only provide compositional information on samples but also assist in identifying adulteration, origin, manufacturer information (Bernal et.al., 1996). Products adulterated with starch-containing substitutes (e.g. cereals or malt), maltodextrins or caramelised sugar exhibit high amounts of free fructose, free glucose and sucrose as well as huge levels of total glucose (Prodolliet and Hischenhuber, 1998). The analysis of free and total carbohydrates in coffee made it easy to detect intentional adulteration of coffee with coffee husks and sticks, moreover it helps to discriminate the adulterated coffee from non adulterated one by analyzing the adulterants nature (Domingues et al., 2014).

Carbohydrate content in green coffee has been studied during the developing of grains and processing of beans and many of the carbohydrates (over 40%) are water insoluble and consist of polymers of mannan, arabinogalactan and cellulose (Arya and Rao, 2007).

Sucrose, which corresponds to about 6–7% dry matter accounts for more than 90% of total low molecular carbohydrates while only small quantities of free reducing sugars are present. Moreover, coffee husks in soluble coffee are detected by the higher content of xylose and mannitol as a tracer of adulteration.

High levels of total glucose indicate adulteration with maltodextrin, caramel, or cereals, while high levels of free fructose may be an indicative of adulteration with chicory (Nogueira and Do logo, 2009). Oliveira et.al described carbohydrate content analysis of coffee by chromatography for specific carbohydrates such as glucose, xylose and fructose are used to quantify adulterants in coffee (Oliveira et al, 2009).

Moreover, there are different methods developed for the identification of adulteration of coffee with chicory and cereals based on the total content of polyalcohols and sugars (Prodolliet and Hischenhuber, 1998). The importance of studying the carbohydrate composition of coffee is to monitor whether non coffee carbohydrates are added, to detect adulterants and to maintain organoleptic attributes of a quality coffee. Plants consisting of enough soluble carbohydrate can adulterate coffee (Arya and Rao, 2007).

3. Materials and Methods

3.1. Location of the Study

The study was conducted at Addis Ababa University, Center for Food Science and Nutrition, Addis Ababa University, Department of Chemistry and Ministry of Agriculture, Coffee quality inspection center laboratory and Jije analytical service laboratory.

3.2. Experimental Design

As investigative materials, Harar coffee was mixed with six rowed barley (*Hordeum vulgare* L.) medium roasted and finely ground at proportions of 5%, 10% and 20% w/w barley, medium roasted & finely ground pure Harar coffee and pure barley were used as a control, medium & dark roasted 50:50 % w/w coffee barley mixture was prepared for GC analysis.

3.3. Raw Materials

Pure *Harar* coffee was provided from FDRE Ministry of Agriculture, Coffee Quality Inspection Center and the six rowed barley was purchased from the local market (Addis Ababa, Ethiopia).

3.4. Raw Material Preparation

Roasting, Grinding and Mixing of Barley & Coffee

The pure Harar coffee was roasted at medium roast in Probat® roaster at 200°C for 70 minutes, dark roasted for 90 minutes and finely ground in Mahlkonig® coffee grinder for 60 s, $0.15 < D < 0.5$ mm. The barley was medium roasted at 250°C for 30 minutes, dark roasted for 32 minutes based on luminosity color standards, the roasted barley was also finely ground in Mahlkonig® coffee grinder for 60 s, $0.15 < D < 0.5$ mm .The roasting and grinding were done according to the study of Reis et al., 2013. The coffee and barley roasting, grinding, packaging and sensory evaluation were carried out at coffee quality inspection center, coffee cupping and liquoring unit. Then, for the study purpose, proportions of 5 to 20% w/w barley to coffee mixtures were prepared intentionally by mixing 95% coffee with 5% barley, 90% coffee with 10% barley and 80% coffee with 20% barley then for the homogeneity of the samples, each of them were mixed thoroughly by a Stuart® electronic mixer (homogenizer), the stirrer wheel and containers were cleaned properly before and after each mixing. Then the roasted & ground pure coffee, pure barley and their mixtures were put into polyethylene bags for the analysis.

3.5. Preparation of Sample and Procedure

3.5.1. Sample Preparation for GC analysis

Pure coffee (medium roasted), coffee barley mixture (50:50% w/w) (medium and dark roasted), pure barley (medium roasted) finely ground were prepared for the GC analysis.

30 gram coffee /barley or mixture was soxhlet exracted with dichloromethane and acetone (1:1 v/v) for 6 hours, the extract passed through anhydrous sodium sulphate then the extract evaporated to dryness, the residue was then dissolved in 2 ml hexane, the contents was passed through silica gel column and eluted with hexane and dichloromethane (DCM) (1:1 v/v) then it was evaporated to dryness, the residue was dissolved in 1 ml of hexane and kept in refrigerator till analysis by GC.

GC analysis

A volume of 1µl of the sample was injected in splitless mode to GC-MS (Agilent Technologies Inc. Santa Clara, USA), Agilent 5890 GC equipped with HP-5ms column ultra inert constant flow, capillary column (30 m×0.25 mm ID, 1µm film thickness) .Helium (He) was the carrier gas at a flow rate of 1.0 ml/min., full scan mode, that revealed the total ion current (TIC) chromatograms.

Injection volume 1µl, splitless mode

A valve heater temperature 280^{o}C

Oven temperature

	Rate	Valve	Hold time	Run time
	°C / min	°C	min.	min.
Initial				
Temperature		60	2	2
Ramp 1	3	240	5	67

Column HP-5ms ultra inert

Constant flow

Inlet temperature 260^0C, SCAN mode

Acquisition and data processing were performed using Chem Workstation software and NIST 14 L.

3.5.2. Infrared (IR) Spectroscopy Analysis

Near infrared analysis was carried out to identify the functional groups on coffee and coffee mixture surface. The coffee or coffee mixture was ground as fine powder with minimal moisture content then 1mg of the coffee barley mixture was mixed with 100 mg potassium bromide (KBr), analytical grade and the mixing was carried out in small pistil and mortar, after a thorough mixing, it was put in Perkin Elmer die for compressing the sample to form pellets using a pressure for 5 minutes, a thin transparent pellet was formed. The spectra were recorded in KBr pellets using IR spectrophotometer, the pellet was put in a confiner and placed in the IR compartment. Then the spectra were measured on a double UV-VIS -NIR spectrophotometer, Perkin Elmer lamda in 4000 cm^{-1} to 400 cm^{-1} with 32 scans with a resolution of 2 cm^{-1} through transmission IR measurement (Pujol et. al., 2013). Then the graph was plotted using Microcal Origin® 6 software.

3.5.3. Trained Panelists

Six expert tasters, three males and three females aged 30 to 55, who are very experienced on coffee quality control were recruited from coffee quality inspection center and trained. The training was short to measure each assessor is consistent in their own score (Kemp et al. 2009). The sensory evaluation was carried out in coffee quality inspection center cupping and liquoring unit laboratory, sensitivity of the panelists was trained by profile testing for five sessions for profile analysis and used the terms that best describe the samples described by Varvolgyi et al., 2013 then agreement protocol on descriptive terms was reached and then the intensity scale test was discussed and also reference sample 100% w/w Harar coffee was presented.

QDA®, Quantitative Descriptive Analysis of the coffee barley mixture was assessed by using unstructured scale in triplicate (Varvolgyi et al., 2013; Manzocco and Lagazio, 2008) and the reference sample was presented along with test samples. The QDA method was adopted anchored at 1.25 cm as low and 13.75 cm as high in intensity of respective attributes, the perceived intensities of each of the attributes was indicated by cutting the scale by a vertical line on a score card of a particular code number. The length of the line from left end and up to the point of cutting indicated the intensity of the attribute (Prakash et al., 1998).

For each sample of coffee barley mixture brew was prepared by putting 5.5g coffee or coffee barley mixture in 100ml water just off the boil (Oeistreich-Janzen, 2010), stirred for 5 minutes and then filtered and cooled to a temperature of 55^{o}c as serving temperature and three digit coded samples were presented monadically in triplicate to the panelists with 15 ml in 100 ml white porcelain vessel (Varvolgyi et al., 2013). The order of presentation of the samples was 20% barley, 5% barley and then 10% barley w/w mixed with Ethiopian Harar coffee with three digit coded samples (Stone and Sidel, 1993) as 734,568 and 216 respectively. Pure Ethiopian Harar coffee roast and ground brew was used as a reference sample and served with the samples to the trained panelists. Room temperature drinking water was presented with expectoration cup .The sensory evaluation room was also quite and illuminated with natural light through the window. The scale is 15 cm long and anchored scale 1(detectable) to scale 10 (very intense) (Varvolgyi et al., 2013).

3.5.4. UV Spectroscopy

Sample preparation for UV absorbance measurement (Wanyika et al., 2010; Komes et al., 2009; Siripat et al., 2009)

0.25 g roast and ground Ethiopian Harar coffee or mixture with barley was weighed and dissolved in distilled water to net volume of 20 ml.

The 20 ml sample solution were pipetted to 250 ml flask and 10 ml of 0.01 mol/l HCl, 2 ml of basic lead acetate solution were added and then made to the mark with distilled water, and then it was shaken up and filtered to clarify using Whatmann ®filter paper.

50 ml of the filtered solution was pipetted and added to 100 ml flask then 0.2 ml 4.5 mol/l H_2SO_4 was added and was made to the net volume with distilled water then the flask shaken up and filtered to clarify using whatmann® filter paper.

Then the absorbance of the samples was measured on UV spectrometer (Perkin-Elmer lambda 19, wavelength range 170 nm to 3200 nm, double monochromator) lambda at 274 nm using 10 mm quartz cuvette. Then the absorbance measurement of pure and adulterated coffee samples were recorded and the caffeine levels (ppm) of the samples were calculated from the regression equation of the best line of fit of the standard. Measurements were in triplicates.

3.5.5. Total Carbohydrate by Difference

Determination of moisture content

The moisture content of the coffee and coffee barley mixture was determined by oven drying method of AOAC 925.10 (2000)

Empty aluminum vessels and lids were dried using drying oven for 30 minutes at 105 ^{o}c, then the cups and lid transferred to a dessicator containing silica gel, then cooled for 30 minutes and was weighed. 5 gm of the coffee or coffee mixture was transferred to dried and weighed vessels. The coffee or coffee mixture containing vessels were dried in an oven for 3 hours at 105 ^{o}c , then the vessels along with the coffee or coffee mixture cooled in a dessicator at room temperature and reweighed. The following equation was used to determine moisture content.

$$\% \text{ moisture} = \frac{\text{weight of wet sample - weight of dried sample x } 100}{\text{Weight of wet sample}}$$

Determination of total ash content

To determine the total ash content of coffee or coffee mixture dry ashing method of AOAC 930.22 (2000) was used.

In a small white porcelain 2 g of coffee or coffee barley mixture was put and heated on a hot plate confined in a fume hood, until the smoke ceases and the sample thoroughly charred the temperature was steadily increased. The porcelains containing the samples were put into a muffle furnace at 550 ^{o}C for 4 hours. Then sample containing porcelains were moved out of muffle furnace and put in a dessicator for an hour.

The amount of ash is determined by the equation

$$\% \text{ Ash} = \frac{W_2 - W_1}{W_3} \text{x } 100$$

W_1=Weight of empty crucible

W_2=Weight of crucible +Ash

W_3=Weight of original sample

Determination of crude fat content

The crude fat content of the coffee or coffee mixture was determined by soxhlet method, according to AOAC 935.38 (2000)

2 g of coffee or coffee mixture was put into a porous cellulose extraction thimble and the thimble was covered with non fat cotton wool. The thimble was suspended above the vessel containing 50 ml diethyl ether, the solvent and underneath the condenser. Into the vessel dried at 105^{o}c in drying oven, 50 ml diethyl ether and boiling chips were added and put in the extraction chamber and heated at 55^{o}c and then the solvent evaporated and moved up into the condenser and converted into a liquid that goes into samples in extraction chamber. Then the solvent moved covering the samples and eventually the solvent moved to the vessels carrying the lipids. After 3

hours of extraction process, the vessel containing the solvent and the lipid was removed from the extraction chamber and the solvent was evaporated and the mass was quantified gravimetrically.

The percentage of crude fat in initial sample is calculated as

$$\% \text{ crude of fat} = \frac{\text{weight of fat}}{\text{Weight of sample}} \times 100$$

Determination of crude protein

Crude protein of coffee or coffee barley mixture was determined by Kjeldahl method, according to the method of ES ISO 1871:2013.

During digestion, 0.5 g of coffee or coffee barley mixture was put in a digestion flask, then mixed catalysts of 5 g potassium sulphate and 1 g of copper sulphate were added onto the flask. Then 6 ml concentric sulphuric acid and 3.5 ml of 30 % hydrogen peroxide were also put in the flask. Then the digestion continued for three hours until frothing stops. Then the samples are cooled.

Distillation, 50 ml distilled water and 25 ml 40% sodium hydroxide were added to the digested sample in the flask then the flask was put into distillation apparatus. To the receiving flask, 25 ml distilled water, 25 ml boric acid and 5-7 drops of methyl red were added. The distillation process was ceased when the volume of the receiving flask reached between 200 to 250 ml.

Eventually, titration was carried out to determine the nitrogen content by titration of the formed ammonium borate with 0.1N Hydrochloric acid.

$$\% \text{ of Nitrogen} = \frac{\text{N HCL x}(V_{\text{sample - }} v_{\text{blank}}) \text{ Lx14 g / mol x 100}}{\text{g of sample}}$$

$$\% \text{ Protein} = 6.25 \times \% \text{ N}$$

Determination of total carbohydrate by difference

The total carbohydrate content of the coffee & barley mixture was determined by difference, by subtracting the sum of percentage of crude protein, crude fat, crude ash and moisture from 100.

% total carbohydrate = 100 - (% Moisture + % Fat+ % Protein + % Ash**)**

3.5.6. p^H Measurement of the Brew

The taste of coffee is largely dependent on the P^H of coffee brew (Belitz, et al., 2009). Meanwhile (Gloess et al., 2013) stated as there is no correlation between p^H of coffee brew and perceived acidity in flavor during sensory evaluation, however the addition of roasted ground barley in pure coffee affects the optimum flavor p^H of Arabica coffee, p^H measurement of coffee barley mixture brew is used as a complementary technique of adulteration detection (Fontes et al, 2001) by sensory evaluation acidity measurement with intensity scale.

2 grams of medium ground coffee barley were accurately weighed into 200 ml glass bottle and 100 ml of distilled water was added then the glass bottle was boiled for 10 minutes and the mixture was filtered through Whatman® qualitative filter paper then 50 ml of the filtered extract was used for p^H value determination by p^H 600 meter (Wang and Lim, 2012). Measurements were done in triplicates.

3.7. Caffeine Analysis Curve Preparation

Stock solution of 1000 ppm was prepared by dissolving 100 mg pure caffeine in 100 ml distilled water then working solutions of 0 ppm, 10 ppm, 20 ppm, 40 ppm, 60 ppm and 80 ppm were put in 25 ml volumetric flask and 1ml hydrochloric acid was added before adding distilled water to the top mark of 25 ml volumetric flask. Then the calibration curve was drawn by using linear regression analysis of the concentrations of the standard solution versus absorbance values. UV spectrometer (Perkin-Elmer lambda 19, wavelength range 170 nm to 3200 nm, double monochromator) were used.

3.8. Data Analysis

Data obtained from proximate, caffeine analysis by UV spectroscopy, p^H measurements were analyzed as one way ANOVA and significance of means were declared at $p<0.05$ and mean separation was carried out with Least Significant Difference(LSD) comparison meanwhile sensory attributes were evaluated using one way ANOVA considering main effect and blocking judge effect. SPSS software version 20 was used for the statistical analysis and significance of means were declared at $p<0.05$ and mean separation was carried out with Duncan multiple comparison procedures of the SPSS. Calibration graph, spider plot were drawn using Microsoft Office Excel 2007. Graphs for IR measurements were plotted using Microcal Origin-6 software. The spectral data of the samples were compared with NIST 14L.GC-MS spectra library.

4. Results and Discussion

4.1. Peak Identification from GC analysis

This study found peaks which were detected in pure barley but not found in pure coffee chromatograms with quality match of > 90 % with reference to NIST 14.L; The compounds identified were 3-Eicosene (E)-,retention time 30.759 min.; E-15-Heptadecenal,retention time 27.384 min.; 2,4-Di-tert-butyl phenol, retention time 22.121 min. and 2-tetradecene (E)-, retention time 19.503 min.; These compounds could have a potential to be chemical markers for detecting the adulterant barley as they were detected in 50:50% wt. coffee barley mixtures, both medium and dark roasted. Hovell et al. (2010) stated that the whole chromatogram, larger peaks and small peaks could have important roles in discrimination of coffee variety consequently this study identified peaks with higher quality match and less area percentage on the chromatogram but crucial to detect the adulterant barley in roasted and ground coffee.

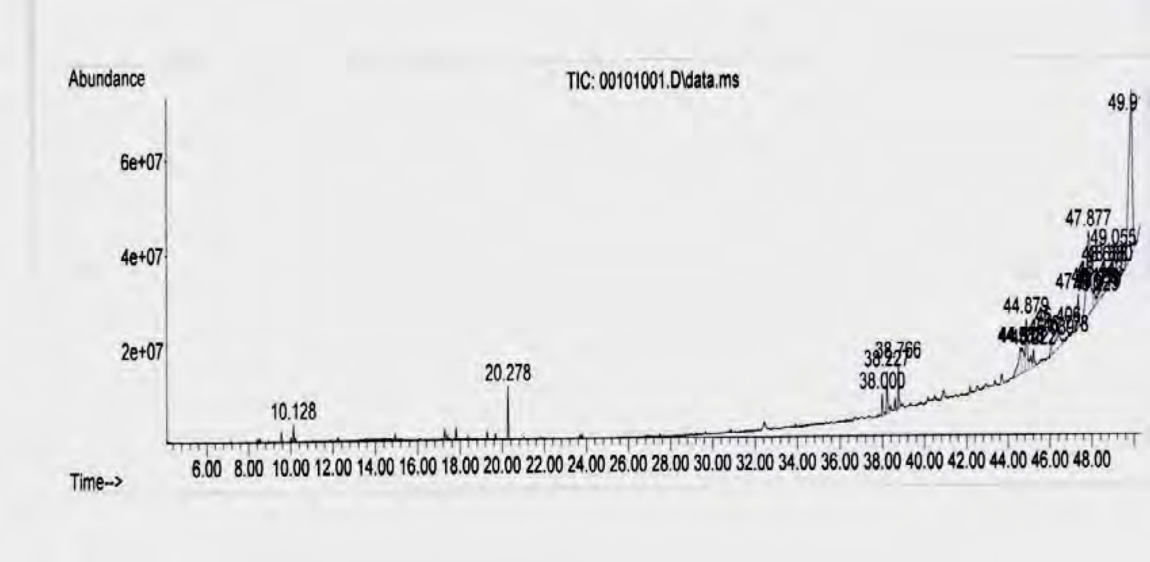


Fig.4.1 GC chromatogram for medium roasted pure coffee

Table 4.1 compound identification from GC peak analysis

Retention time (R.T)	compound	Area %
	Roasted coffee	
49.912 min	*3H-pyrazol-3-one,2,4-dihydro- 5-(4-methyl-1-piperidinyl)-2-phenyl-	32.117%
47.877 min	*Acetamide , N-(4-fluorophenyl)-2,2,2-triflouro	18.948%
48.638 min	*Tris(tert-butyldimethylsilyloxy) arsane	5.461%
44.879 min	*Anthracene, 9,10—dihydro-9,9,10-trimethyl	5.011%
46.406 min	*Octasiloxane**,** 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15 -Hexadecamethyl	4.593%
49.055 min	*Octasiloxane**,** 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15 -Hexadecamethyl	4.578%
44.588 min	*9H-Fluorene-4-carboxylicacid,9-oxo-,	4.538%
48.880 min	*Tris(tert-butyldimethylsilyloxy) arsane	3.993%
44.676min	*Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13 -tetradecamethyl	3.300%
38.766 min	*Bis (2-ethylhexyl) phthalate	2.445%
38.227 min	*2-(2-hydroxyphenyl)buta-1,3-diene	2.375%
20.278 min	Caryophyllene	1.905%
47.376 min	*Octasiloxane**,** 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15 -Hexadecamethyl	1.718%
48.443 min	*Octasiloxane**,** 1,1,3,3,5,5,7,7,9,9,11,11,13,13 15,15 -Hexadecamethyl	1.407%
45.222 min	*3-Quinolinecarboxylic acid, 6,8-di fluoro-4 hydroxy-,ethyl ester	1.347%
46.089 min	*Ethane, 1-(4,4,4-triflouro-1,3-dithiobutyl) -2-(3, 3,3-triflouro-1,2-dithio propyl	1.347%
38.000 min	*1H-benzimidazole,5, 6-dimethyl	1.122%

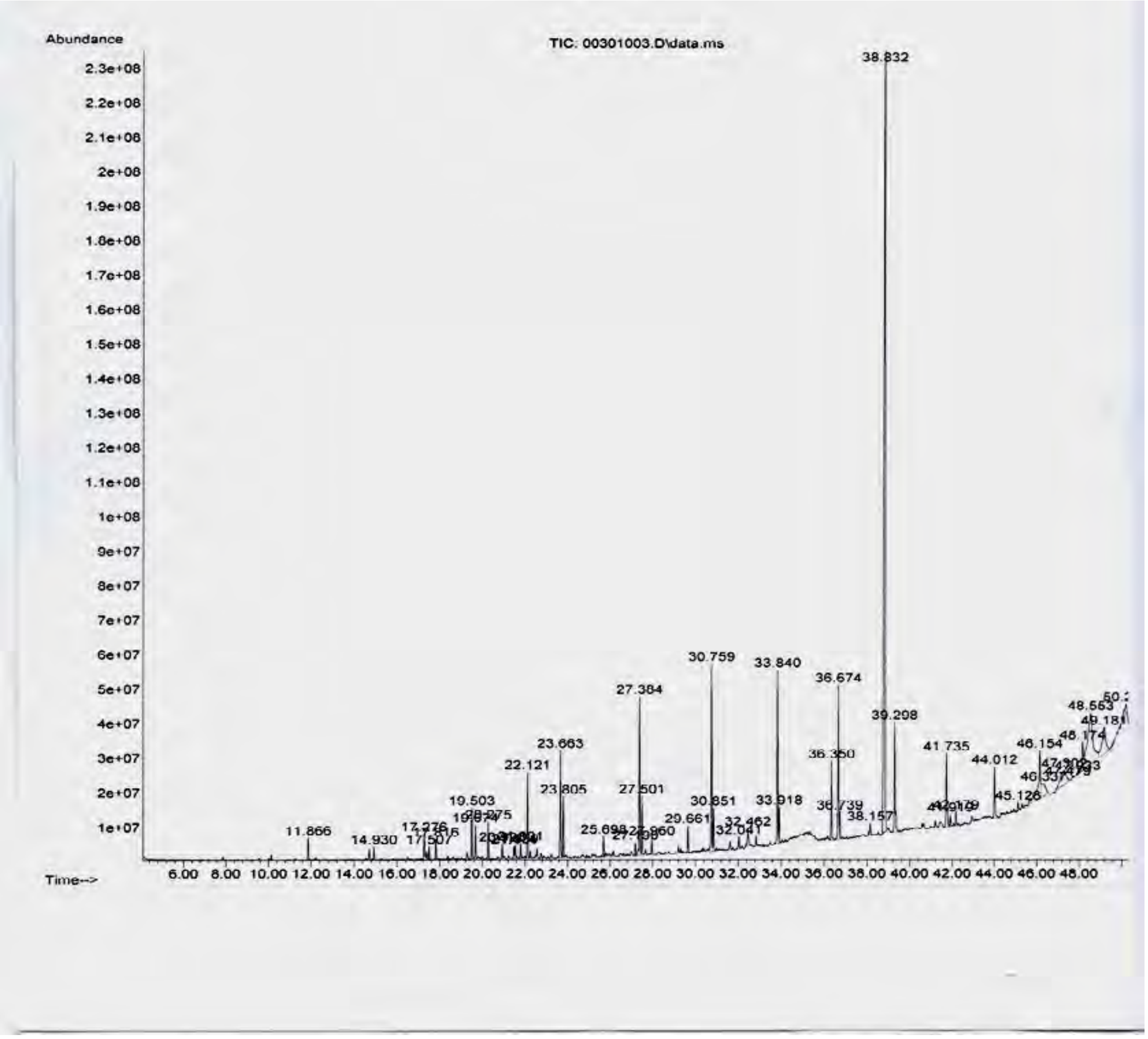


Fig.4.2 GC chromatogram for medium roasted pure barley

Retention time (R.T)	Compound	Area %
	Roasted barley	
38.832 min	*Diisooctyl phthalate (quality match, 11)	33.307%
48.553 min	*1, 2-Bis (trimethyl silyl) benzene	9.139%
30.759 min	3-Eicosene (E) – (quality match, 96)	4.216%
33.840 min	n-Tetracosanol-1	4.048%
46.154 min	1-Nonadecene	3.873%
36.674 min	1-Docosene	3.611%
27.384 min	E-15-Heptadecenal (quality match, 99)	3.595%
49.181 min	*Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13	

	-tetradecamethyl	3.395%
39.298 min	13 methyl-Z-14-Nonacosene	3.093%
23.663 min	Cetene	2.462%
47.302 min	*Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13 -tetradecamethyl	2.420%
41.735 min	13 methyl-Z-14-Nonacosene	2.356%
22.121 min	2,4 –Di- tert –butyl phenol (quality match,97)	2.072%
36.350 min	* [1,1’-Biphenyl]-2,3’-Diol	2.047%
44.012 min	1-Nonadecene	1.685%
46.337 min	*1H-Indol-2-carboxylicacid , 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4, 5,6, 7-trihydro-, isopropyl ester	1.603%
48.174 min	*1, 2-Bis (trimethylsilyl) benzene	1.306%
23.805 min	Hexadecane	1.239%
27.501 min	Octadecane	1.237%
30.851 min	Eicosane	1.101%
19.503 min	2-Tetradecene, (E) – (quality match, 99)	1.046%

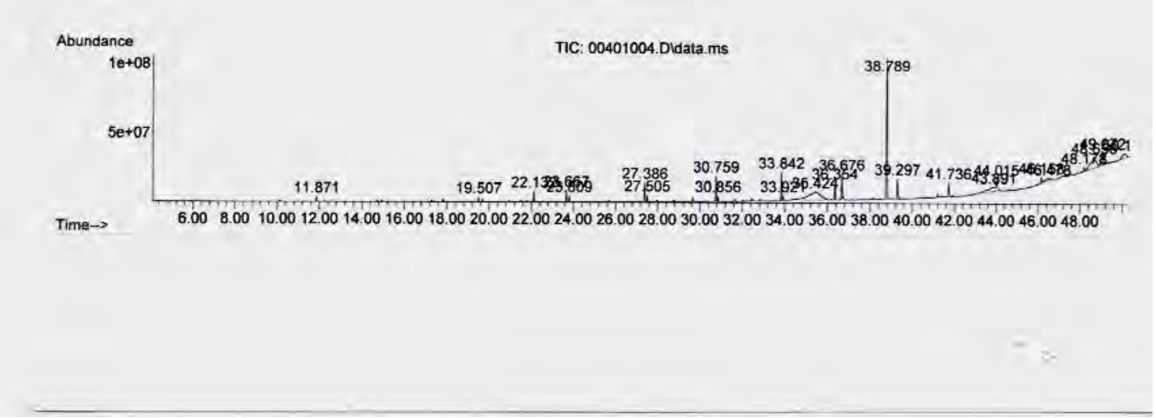


Fig.4.3 GC chromatogram for medium roasted coffee barley mixture (50:50 % wt)

Retention time (R.T)	compound	Area %
	Barley coffee mixture	
	Medium roast	
38.789 min	*Diisooctyl phthalate (quality match, 62)	23.253%
35.424 min	*Silane,dimethyl (2-naphthoxy) heptyloxy	16.658%
48.658 min	*Tris (tert-butyldimethylsilyloxy) arsane	11.672%
43.891min	*Hexadecanoic acid, Octadecyl ester	6.692%
49.072 min	*Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13 -tetradecamethyl	3.735%
33.842 min	1-Nonadecene	3.649%
30.759 min	3-Eicosene (E) -	3.535%
50.137min	*Tetrasiloxane,decamethyl	3.332%
39.297 min	1-Nonadecene	3.167%
44.015 min	17-Pentatriacontene	3.155%
36.676 min	Octacosyl acetate	2.780%
27.386 min	1-Octadecene	2.551%
41.736 min	13 methyl-Z-14-Nonacosene	2.275%
36.354 min	*Benzenamine,4, 4’-[(1-methylethylidene) bis(4,1-phenyleneoxy)] bis-	2.257%
46.478 min	*Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13 -tetradecamethyl	2.032%
23.667 min	Cetene	1.393%
22.132 min	Phenol 2, 5-bis (1,1-dimethylethyl)	1.372%
46.158 min	Tetrasiloxane, decamethyl-	1.256%

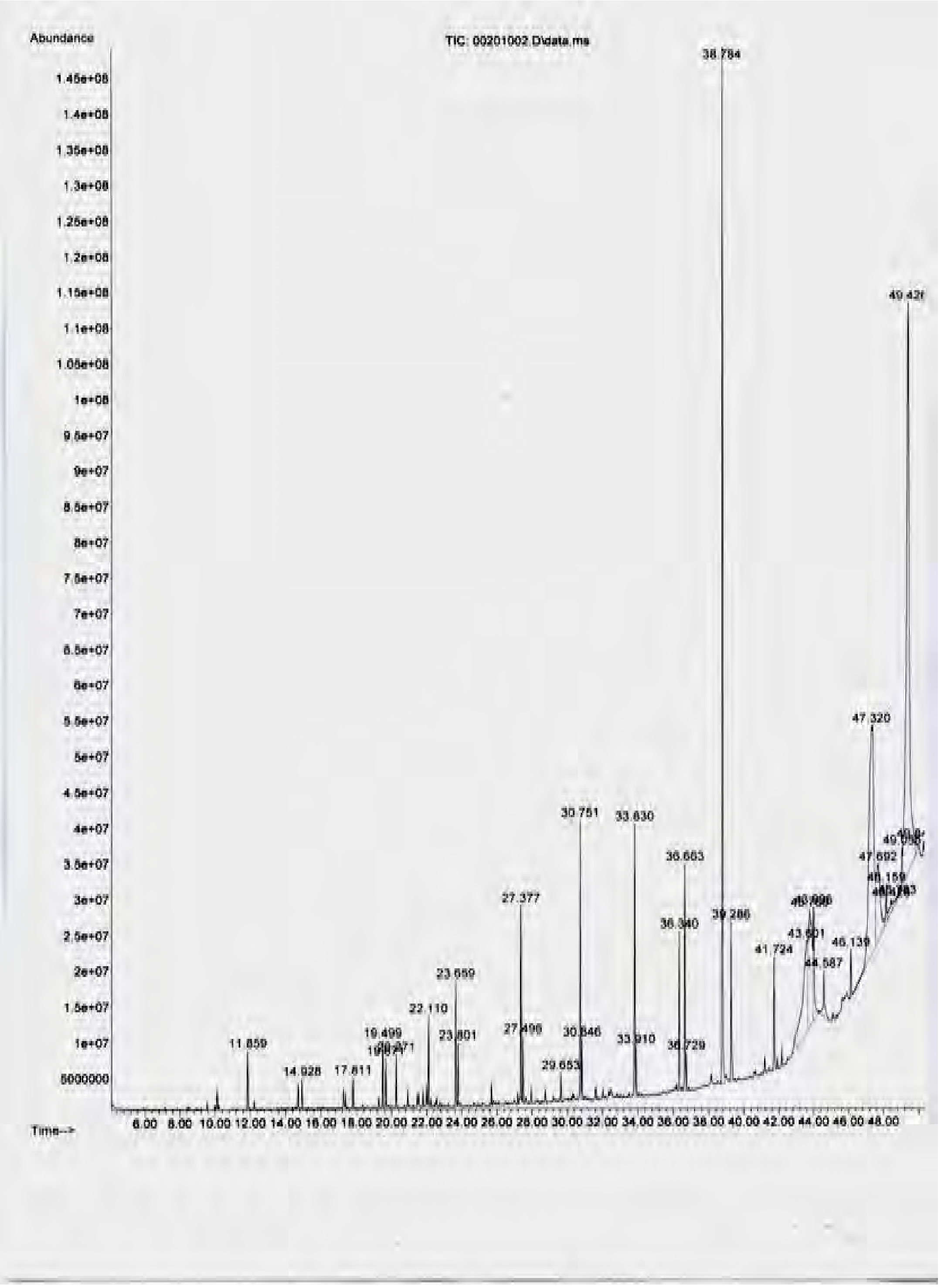
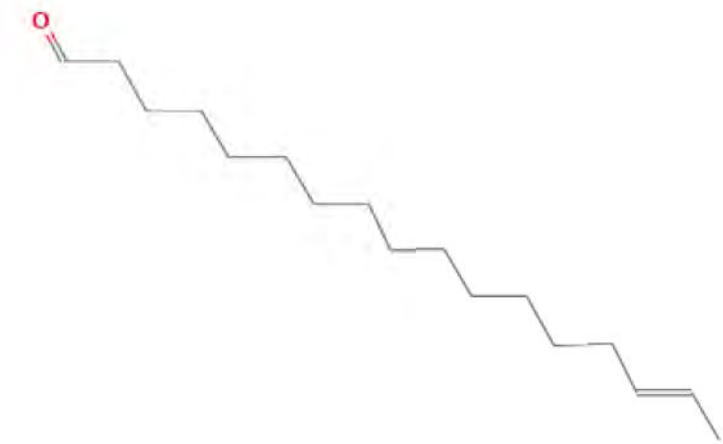


Fig.4.4 GC chromatogram for dark roasted coffee barley mixture (50:50 % wt)

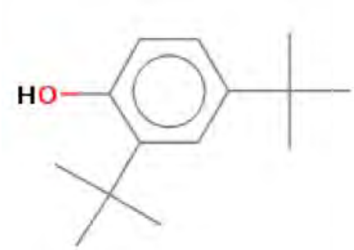
Retention time (R.T)	compound	Area %
	Barley coffee mixture	
	Dark roast	
49.426 min	*Hexadecanoic acid, Octadecyl ester	25.562%
47.320 min	*Hexadecanoic acid, Octadecyl ester	16.983%
38.784 min	*Diisooctyl phthalate (quality match,43)	11.974%
43.601 min	*Hexadecanoic acid, Octadecyl ester	7.144%
43.769 min	*Hexadecanoic acid, cyclohexyl ester	6.823%
47.692 min	*4-Flouroaniline, N-Pentafluoropropionyl	5.289%
33.830 min	n-Tetracosanol-1	2.439%
30.751 min	3-Eicosene, (E) -	2.386%
43.996 min	Nonacos-1-ene	2.140%
36.663 min	Cyclotetracosane	2.082%
39.286 min	Cyclotetracosane	1.800%
27.377 min	1-Octadecene	1.754%
36.340 min	*Perazine [1, 1’-Biphenyl]	1.427%
41.724 min	Triacontyl acetate	1.248%
23.659 min	Cetene	1.084 %

[*] compounds having quality match of less than 90 based on NIST 14 library search report

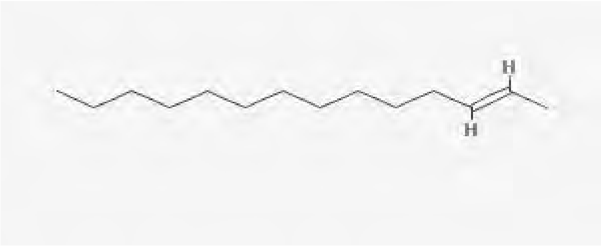
3-Eicosene (E) - ,$C_{20}H_{40}$



$C_{17}H_{32}O$, E-15 Heptadecenal



2, 4 Di tert butyl phenol, $C_{14}H_{22}O$



2-tetradecene (E)-, $C_{14}H_{28}$

Bianchi et al. (2007) on their GC-MS qualitative characterization of roasted barley detected the aromatic hydrocarbon (trimethyl benzene), the phenol (2,4 dimethyl phenol) and the alkane (tetradecane) in roasted barley, this study was also in agreement with the result of Bianchi et al.

Cramer et al. (2005), identified decanal as one of the key odorants found in barley and this study also found out that 2-tetradecene and E-15 Heptadecenal were identified in roasted barley but not in roasted coffee.

Dong et al. (2013) described that the polyunsaturated fatty acids, including linolenic and eicosenoic acids in barley, representing about 60% of the fatty acids engender the lipid oxidation to produce high level of aldehydes and Cramer et.al.,2005 stated that eicosenic acids were detected in barley and unsaturated fatty acids were found as 76.8 to 82.3% of total fatty acids and lipoxygenases and hydroperoxide isomerases were responsible for the oxidation of unsaturated fatty acids consequently forming volatile compounds in barley. Moreover, Cramer et.al. also emphasized that most odorants of barley arise most probably from lipid oxidation ;thus this study identified 3-Eicosene,(E)- in roasted barley (4% of peak area).

Dong et al, (2013) also stated that hexanoic acids are organic acids which were formed in the malting process but which vanish in roasting treatment and this study found hexadecanoic acid, octadecyl ester to be the major peaks in high abundance in the dark roasted coffee barley mixture. Hovell et al. (2010) found hexadecanoic acid, methyl ester as one of the discriminative compounds between Arabica and Robusta coffee varieties.

Bianchi et.al.(2007) detected dibutyl phthalate and dihexyl phthalates in roasted barley GC-MS analysis and this study found diisoctyl phthalate to exist in the GC-MS chromatogram of pure barley and mixtures in greater abundance but the phthalate, Bis (2-ethylhexyl) phthalate was detected in much less abundance in pure coffee.

4.2. Infrared (IR) Spectra Analysis

The qualitative transmission measurement of roasted and ground Harar coffee & coffee barley mixtures 5 to 20 % w/w barley showed that the infra red spectra among 5 % to 20 % w/w barley addition to coffee were not resolved though it was possible to identify the peak regions useful to discriminate the roasted barley from roasted coffee IR spectra using caffeine molecule, lipid content and starch presence ; this might be due to the amount of sample (1 mg) used to form pellets for IR analysis using potassium bromide since the PerkinElmer spectrophotometer clearly resolved the pure roasted barley and coffee IR spectra but not the mixtures (5 % to 20 % w/w) the result obtained is in close agreement with the study of Varvolgi et al. (2013), showed in their study that diffuse reflectance near infra red spectroscopy could not discriminate 1%, 5%, 10% w/w barley except slight variation in 20 % w/w barley. Gholizadeh et al. (2014) described that the total carbohydrate IR absorbance peak in barley was within the range of 1180-950 cm^{-1}.Moreover,Welna et al., (2013) stated that the peak between 1150-900 cm^{-1} in roasted coffee products were attributed to C-O-C group of polysaccharides, C-O stretching at 1070 $cm^{-1}$and 1060 cm^{-1} .

Varvolgi et al., (2013) found out high discrimination power of NIR spectroscopy in addition of barley 49-100% w/w barley in Robusta coffee. Welna et al. (2013) identified the peak at 1150 cm^{-1} to be attributed to bulk carbohydrates in chicory, which could be the most significant in discriminating pure coffee and adulterated ones (Welna et al. 2013).

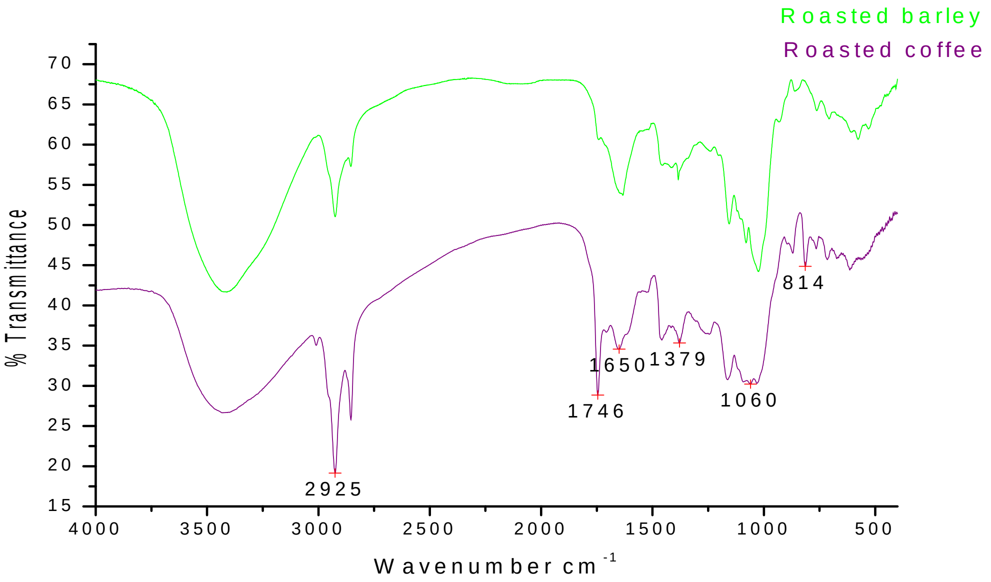


Fig.4.5 IR spectra of medium roasted pure coffee & barley

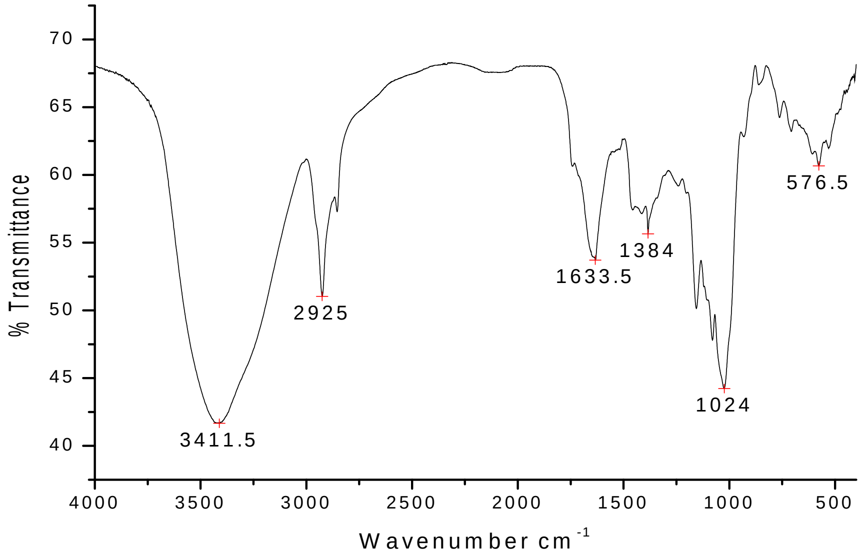


Fig. 4.6 IR spectra for medium roasted pure barley

The sharp peak at 2925 cm^{-1} in roasted coffee was more intense than the sharp peak at 2925 cm^{-1}in roasted barley. Reis et al., 2013 showed that this peak at 2925 cm^{-1} was attributed to C-H stretching of lipids due to higher lipid content in coffee than barley. Moreover, as it can be seen on the IR spectra, roasted barley was devoid of a band at about 2852 cm^{-1} next to the sharp peak at 2925 cm^{-1}, the band at about 2852 cm^{-1}was attributed to C-H stretching of methyl (CH_3) group in the caffeine molecule and the peak was not observed in roasted barley IR spectra since barley has no caffeine in it. These two absorption bands were affected by lipids and caffeine in the roasted coffee but only by lipids in roasted barley that influence the absorption bands (Reis et al., 2013).The result of the study was in agreement with Reis et al., 2013.

The IR absorption region 2250 cm^{-1} to 1850 cm^{-1} was identified to be discriminative of the adulterant barley from the roasted coffee according to the study of Reis et al., 2013, this might be due to high amount of phenolics attached to non degraded starch or it might partially be associated to non degraded starch's hydration water effect (Reis et al., 2013).this study also found out that the region between 2250 cm^{-1} and 1850 cm^{-1}could be used to discriminate roasted barley from roasted coffee IR spectra. Moreover, the absorption band at 1746 cm^{-1} in roasted coffee was not evident in roasted barley IR spectra, Reis et al., 2013 also found out that the band 1745-1742 cm^{-1} was weak in roasted barley due to its low lipid content, the band was formed by the C=O vibrations of triglyceides and aldehydes. This study also found that the band at 1650 cm^{-1} in roasted coffee was not noticed in roasted barley IR spectra. Reis et al. described that decaffeinated coffee samples has shown less absorbance in the range 1700-1600 cm^{-1}.

According to the study of Reis et al. the absorbance band region 950-700 cm^{-1} was identified as beneficial in the discrimination of roasted coffee from its adulterants due to the fact that coffee has no starch but barley does. This difference in the band region could be attributed to the alpha glycosidic links in barley and beta glycosidic links in coffee. The result of this study is in agreement of Reis et al., 2013.

This study found out that the absorbance peak region between 1746 cm^{-1}and 1650 cm^{-1}in roasted coffee IR spectra had been absent in the IR spectra of roasted barley, with only a peak at 1643 cm^{-1}, Welna et al., 2013 stated that the peaks at 1700, 1658 and 1600 are attributed to the caffeine molecule and the absorbance band at 1643 cm^{-1} were due to oxalate. The result of the study is in agreement with Welna et al., 2013.

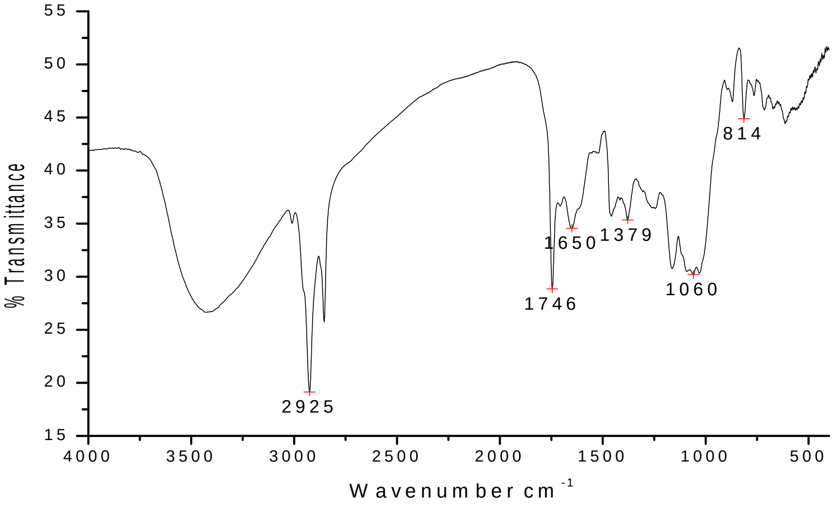


Fig.4.7 IR spectra for medium roasted pure coffee

Gholizadeh et al., 2014 stated that barley IR spectrum 1180-950 cm^{-1} was attributed to total carbohydrate peak moreover, there are other peaks of barley responsible for stretching of N-H and O-H,C-H,C=O carbonyl, amide I,II and for cellulosic compounds.

The peaks and absorption bands found in roasted pure coffee were 3433 cm^{-1},3010 cm^{-1},2925 cm^{-1},2855 cm^{-1},1746 cm^{-1},1650 cm^{-1},1458 cm^{-1},1379 cm^{-1},1246 cm^{-1},1094 cm^{-1},1060 cm^{-1},1030 cm^{-1} and 869 cm^{-1}.

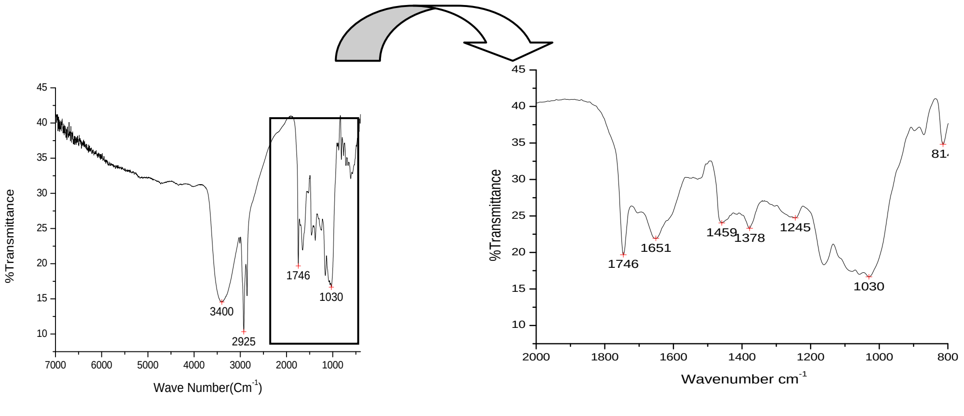
Reis et al. (2013) stated that sharp bands at 2924-2925 cm^{-1} and 2852 cm^{-1} along with absorptions at 1715-1745 cm^{-1} and 760 cm^{-1} were found in both roasted coffee and barley.

Wang and Lim, 2012 revealed that 2920 cm^{-1} in pure roasted coffee was attributed for CH_2 assymetrical stretching of methyl groups ,2850 cm^{-1} was related to C-H symmetrical stretching of methyl groups,1739 cm^{-1} was due to C=O stretching of polysaccharides /hemicelluloses and 1660 $cm^{-1}$was due to C=C stretching band of lipids and fatty acids.

This study found that the peak 3433 cm^{-1} in pure roasted coffee shifted to 3419 cm^{-1} in 10% w/w barley and to 3400 cm^{-1} in 20% w/w barley, these might be related to many vibrations of OH groups and also minor influence of NH functional groups (Pujol et al., 2013). Welna et al. (2013) stated also that the broad band between 3420-3375 cm^{-1} in coffee products was assigned to O-H stretching of hydroxyl groups and water besides it was due to N-H stretching in amines I and II.

The band at 1094 cm^{-1} in pure roasted coffee had shown slight change only in 20 % w/w barley with a band of 1084 cm^{-1}.Welna et.al (2013) described as the most significant difference between pure coffee and adulterated ones occurred at 1150 cm^{-1} with respect to bulk carbohydrates in chicory (Welna et.al. 2013), which can also be indicative of the adulterant barley in coffee. Briandet et al. (1996) also identified pure and adulterated freeze dried soluble coffee in the region 900-1100 cm^{-1} and used caffeine and chlorogenic acid as a primary source of variability in FTIR analysis. Reis et al. (2013) described that the sharp peak in the region 950-700 cm^{-1} in roasted barley had shifted to the bands for the pure roasted coffee due to the difference in polysaccharide types present in coffee and barley, the presence of starch in barley, alpha glycosidic link however beta glycosidic links in roasted coffee.

The sharp peaks at 2955 cm^{-1} and 2855 cm^{-1} in pure roasted coffee were attributed to the occurrence of large amount of lipids in coffee and Pujol et al. (2013) also revealed that the peaks were indicative for the presence of methyl and methylene presence, asymmetric and symmetric stretch of C-H bonds in aliphatic chain. Reis et al. (2013) stated that the spectra for roasted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5000
4000
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Wave Number(Cm^{-1})
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1030
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25
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1378
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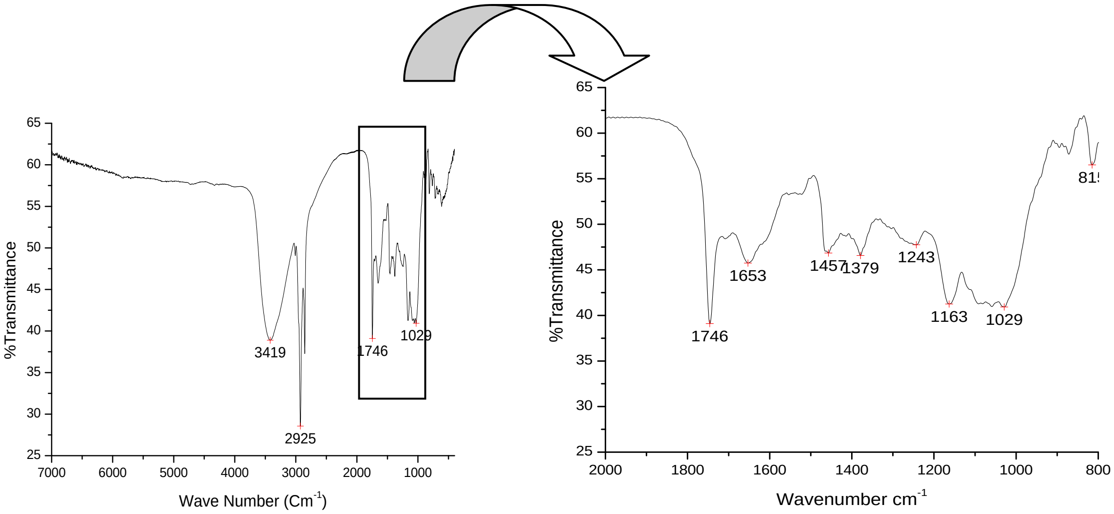
1030
81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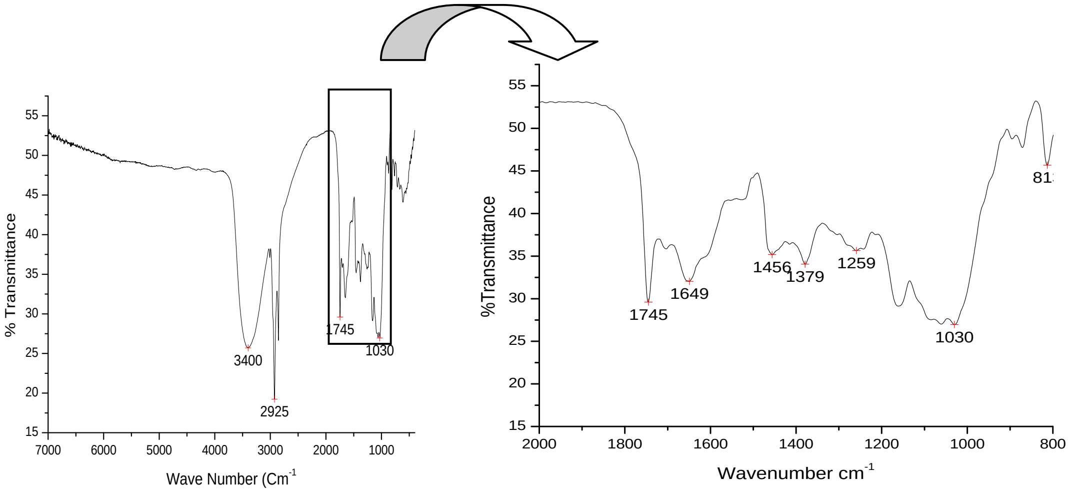
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%Transmittance
Wavenumber cm^{-1}
1745
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1379
1259
1030
81

The IR absorbance peaks and absorption bands in 5% w/w barley were 3400 cm^{-1},3010 cm^{-1},2925 cm^{-1},2855 cm^{-1},1745 cm^{-1},1649 cm^{-1},1456 cm^{-1},1379 cm^{-1},1259 cm^{-1},1162 cm^{-1}, 1092 cm^{-1}, 1061 cm^{-1}, 1030 cm^{-1} and 870 cm^{-1}.

The broad band peak of 5% w/w barley at 3400 cm^{-1} were not similar with the peak for pure roasted coffee 3433 cm^{-1}.

4.3. UV Absorbance Measurement

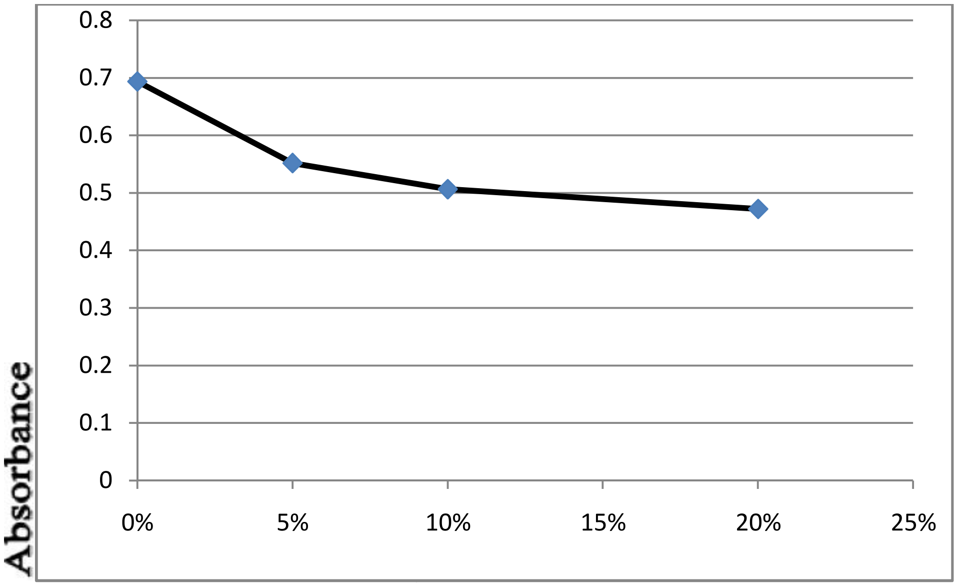
Table 4.2 UV absorbance measurement of pure and adulterated coffee

	100% coffee	5% barley	10% barley	20% barley
UV absorbance	0.6934 ±0.04^{a}	0.5516±0.03^{b}	0.5061±0.03^{b,c}	0.4718±0.07^{c,d}

Means followed by different letters in the same row were significantly different at $p<0.05$

Fontes et al. (2006) stated that among the several approaches to detect adulterants in roasted coffee, the spectroscopic techniques UV-VIS to IR are to be mentioned.

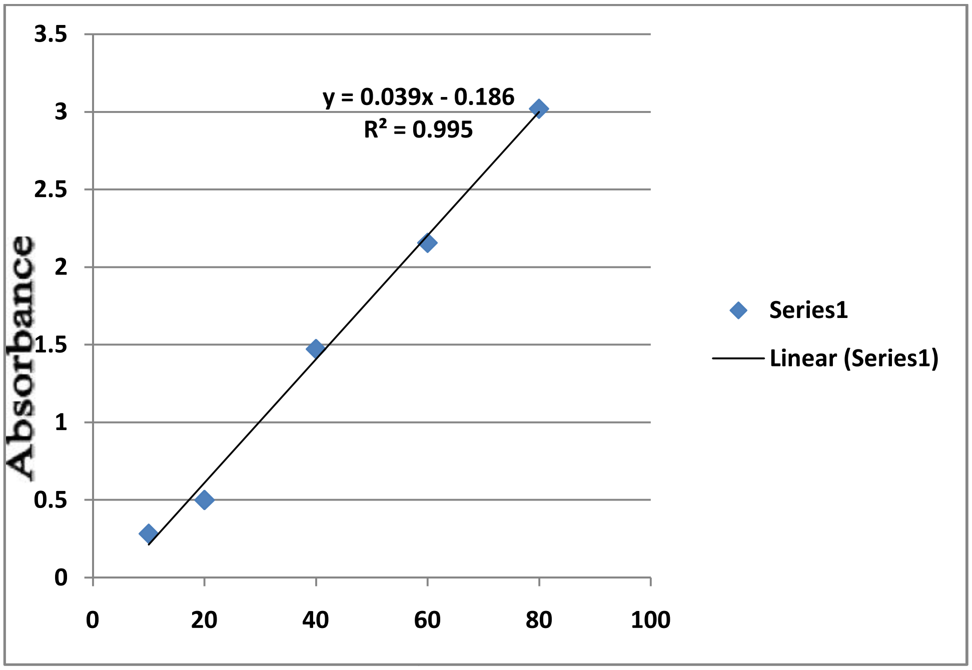
The UV absorbance measurement at 274 nm using UV/VIS spectrophotometer revealed that the result obtained for pure coffee was significantly different from the adulterated coffee (5% to 20% w/w barley) and this study found out that it was possible to detect the adulterant barley as low as 5% w/w barley in roasted and ground coffee.



Concentration (0 to 20% barley w/w)

Fig 4.10 UV absorbance measurement of pure and adulterated coffee

(5 to 20% w/w barley)



Concentration (ppm)

Fig 4.11 Caffeine calibration curve

Souto et al. (2015) used UV-Vis spectroscopy via hot water extraction in 239-405 nm range to discriminate adulterated coffee from pure roasted coffee based on molecular absorption of compounds found in coffee, coffee husks and sticks, this study also detected the adulterant barley in roasted coffee based on the maximum absorption wavelength of caffeine, 274 nm. This result was in agreement with Souto et al. (2015).

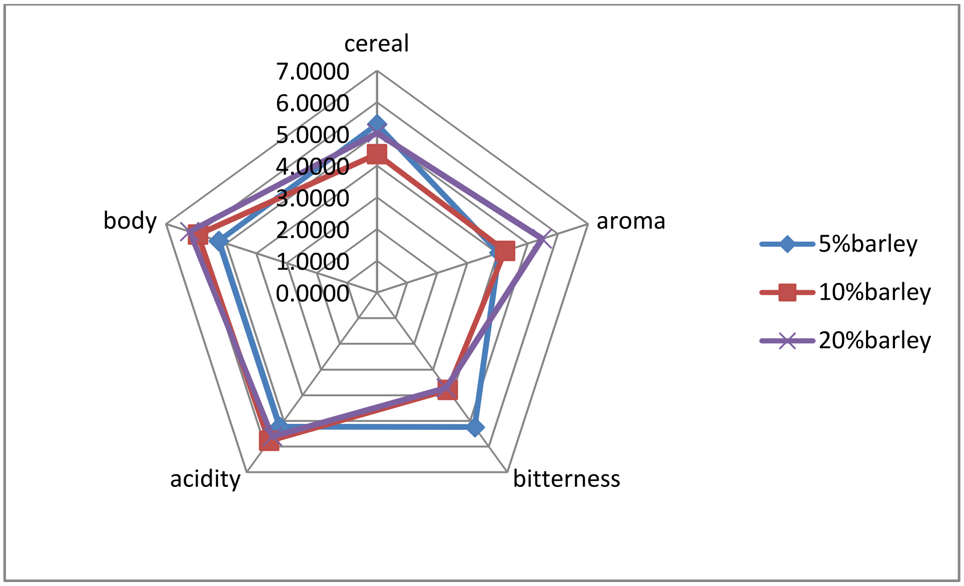
As expected, the absorbance of the coffee barley mixture had decreased from 5% w/w barley addition to 20 % w/w barley since barley does not contain any caffeine (Reis et al., 2013). The result of this study had a potential to be used in adulteration detection of roasted coffee with roasted barley as it enabled the discrimination of low concentration (5% w/w barley) of adulterated coffee from pure roasted coffee, Varvolgi et. al. (2013) and Oliviera et al. (2009) emphasized the necessity of coffee adulteration detection at lower concentrations of the adulterants.

Table 4.3 Caffeine level (mg/L) of pure and adulterated coffee (mean±SE)

	100% coffee	5% barley	10% barley	20% barley
Caffeine (mg/L)	22.55 ± 0.54^{a}	18.91 ± 0.46^{b}	$17.75 \pm 0.48^{b,c}$	$16.87 \pm 0.11^{c,d}$

Means followed by different letters in the same row were significantly different at $p<0.05$

Caffeine is described as good indicator of degree of adulteration hence the results show that caffeine, was significantly ($p<0.05$) decreased from 22.55 mg/L in Ethiopian *Harar* coffee, which is in close agreement with study of Ali et.al, 2012 as found 25.24 ppm in Ethiopian coffee by HPLC method, this method was feasible to detect addition of barley of 5% w/w barley, 10% barley and 20% w/w barley were significantly different from non adulterated coffee sample.


cereal
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aroma
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acidity
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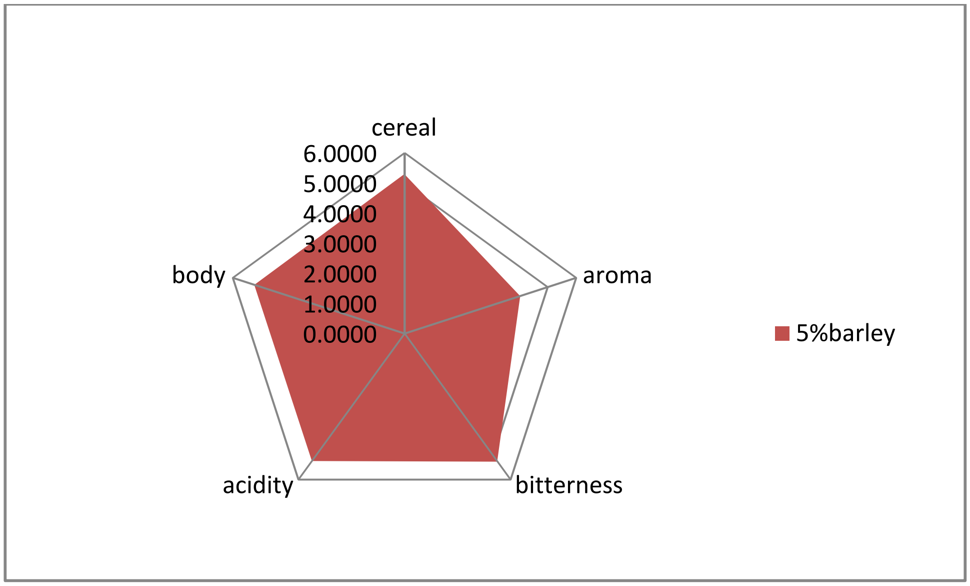
10%barley
20%barley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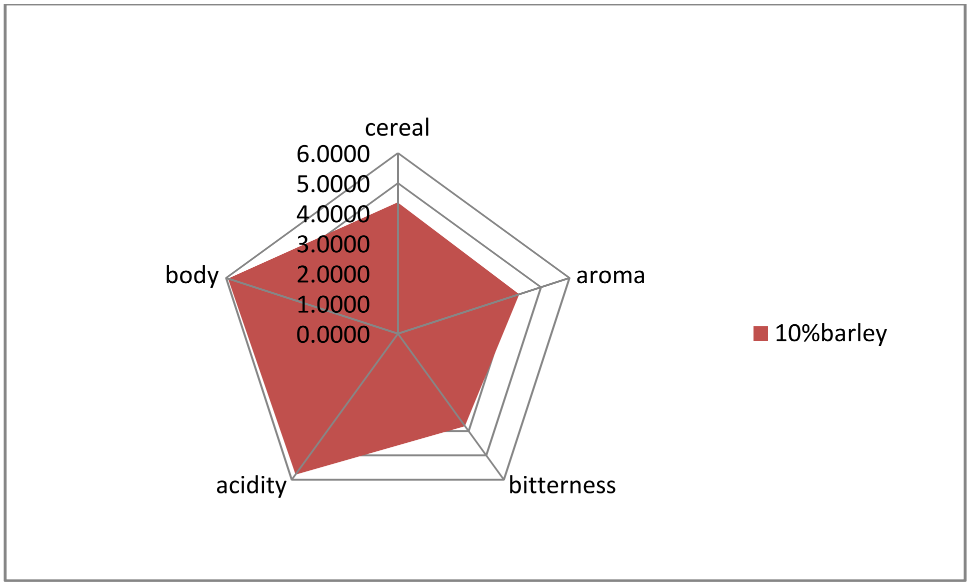
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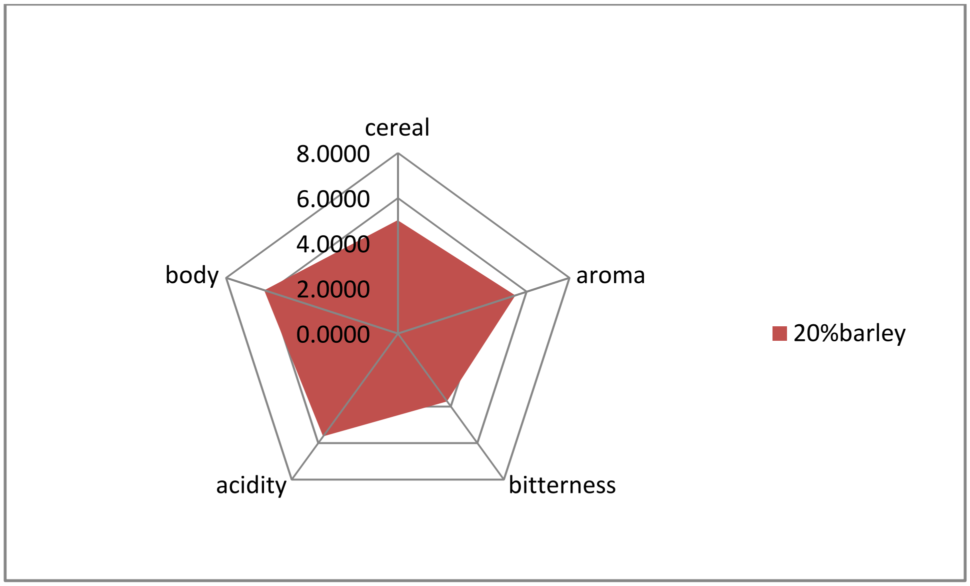
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🟥 20%barley

Table 4.5 Proximate composition of pure coffee and adulterated coffee .Wet basis g /100 g (Mean ± SE)

Parameter	100% coffee	5% barley	10% barley	20% barley
Moisture	2.45 ± 0.01^{a}	3.47 ± 0.03^{b}	3.33 ± 0.03^{c}	3.73 ± 0.01^{d}
Ash	4.69 ± 0.26^{a}	5.69 ± 0.21^{b}	$4.49 \pm 0.007^{a,c}$	$3.32 \pm 0.41^{c,d}$
Fat	14.03 ± 0.09^{a}	$13.55 \pm 0.29^{a,b}$	12.38 ± 0.00^{c}	$12.12 \pm 0.35^{c,d}$
Protein	12 ± 0.05^{a}	$10.13 \pm 0.05^{a,b}$	$9.93 \pm 0.03^{b,c}$	$9.82 \pm 0.015^{b,d}$
Total Carbohydrate	68.71 ± 0.11^{a}	67.16 ± 0.095^{b}	69.87 ± 0.065^{c}	$71.01 \pm 0.780^{c,d}$

Means followed by different letters in the same row were significantly different at $p<0.05$

Moisture content

Addition of barley to coffee significantly ($p<0.05$) changed the moisture content from the pure Ethiopian Harar coffee, the mean moisture content increased with increase in mixture proportion in 5 to 20% w/w barley from pure Harar coffee. The result obtained is in close agreement with that reported by Welna et.al, 2013; moisture content of roast and ground coffee was 0.90-1.56% (Welna et.al, 2013).

Ash content

The ash content of the Ethiopian Harar coffee barley mixture significantly ($p<0.05$) decreased from pure Ethiopian Harar coffee. The increase in Ethiopian Harar coffee barley mixture proportion from 5% to 20% w/w significantly ($p<0.05$) decreased the ash content of 5 to 20%w/w barley from pure Harar coffee. The result obtained for roast and ground coffee is in agreement with Welna et.al, 2013 which was reported as 4.5-4.7%.Whereas the ash content of barley is 2.28-2.55% (Cramer et al., 2005).

Fat content

The fat content of the Ethiopian Harar coffee barley mixture significantly ($p<0.05$) decreased from pure Ethiopian Harar coffee. The free lipid content of barley is 1.99-2.87% (Cramer et al., 2005).

Protein content

As it can be seen from table 1, the protein content of pure Ethiopian Harar coffee significantly ($p<0.05$) decreased with increase in the proportion of Ethiopian Harar coffee barley mixture from 5% to 20% w/w. The protein content of barley is 12.8-16.5% (Cramer et al., 2005).

Total carbohydrate content

As one way ANOVA showed the carbohydrate composition of the barley coffee mixture (10% & 20% w/w) was significantly ($p<0.05$) increased from pure Harar coffee. Meanwhile ash content, fat content and protein content of 5% to 20% adulterated sample significantly decreased ($p<0.05$) from non adulterated coffee sample.

4.6. p^H Measurement

The p^H measurement of Ethiopian Harar coffee and Ethiopian Harar coffee barley mixture showed that there is no significant difference ($p\geq5$) between pure Ethiopian Harar coffee and mixture of Ethiopian Harar coffee with barley and among Ethiopian Harar coffee barley mixtures of 5% to 20% w/w barley. Wang and Lim, 2012 described as acidity, one sensory attributes correlates with high quality coffee and p^H value and perceived acidity of coffee have a linear correlation.Hence the p^H measurement showed similar result of sensory evaluation for perceived acidity. The p^H values obtained were 5.15 for pure Ethiopian Harar, 5.12 for 5% Ethiopian Harar coffee barley mixture, 5.14 for 10% barley and 20 % w/w barley to coffee. The p^H value obtained is in agreement with p^H of acceptable coffee brew, 4.9 to 5.2 described by Belitz et al.

Table 4.6 p^H measurement of Harar coffee and adulterated coffee brews (Mean±SE)

	100% coffee	5% barley	10% barley	20% barley
p^H	5.15 ± 0.01^a	5.12 ± 0.02^a	5.14 ± 0.01^a	5.16 ± 0.03^a

Means followed by different letters in the same row were significantly different at $p<0.05$.

5. Conclusion and Recommendations

5.1. Conclusion

The present study showed that coffee adulteration with roasted barley would have a potential to be detected by gas chromatography analysis and it was observed that 3-Eicosene (E)-, 2-Tetradecene, (E)-, 2, 4 Di tert butyl phenol and E-15-Heptadecenal were identified in roast & ground pure barley, medium & dark roasted barley coffee mixture (50:50 % w/w) but were not detected in pure coffee, roasted & ground.

Infrared spectroscopy could not resolve among 5 % to 20 % w/w barley addition to coffee though it was possible to identify the peak regions useful to discriminate the roasted barley from roasted coffee IR spectra using caffeine molecule, lipid content and starch presence.

UV spectroscopy analysis resulted in discriminating pure Harar coffee from the adulterated coffee as low as 5 % w/w barley addition.

Meanwhile, Sensory evaluation resulted in not discriminating the adulterated Harar coffee samples 5 %, 10 % and 20 % w/w barley despite pure coffee was presented as a reference.

The total carbohydrate determination by difference could be useful in verifying the adulterant barley in roasted & ground Harar coffee, greater or equal to 10% barley addition.

p^H measurement showed that the acidity in the different proportions of adulterated Harar coffee brew, 5 % to 20 % w/w barley, was not different and this study proved similar result for acidity from sensory evaluation using trained panelists.

5.2. Recommendations

In view of the results of the study, the discrimination of barley addition to coffee showed to be feasible using GC analysis, total carbohydrate content and UV spectroscopy analysis.

Therefore, the following recommendations are forwarded:

Since there are multiple adulterants such as other cereal grains liable to adulterate coffee thus detection method for each adulterant's presence in coffee should be studied thoroughly.

Our country possesses different coffee varieties grown in different areas which makes the study of chemical profiles of both green and roasted coffee along with detection of adulterants at lower and higher concentrations with composite coffee of Ethiopia inevitable.

The same as to green coffee beans quality inspection and cup quality assessment, the detection of adulteration of roast and ground coffee quality analysis laboratory should be established along with certification for authenticity.

References

Adapa, P., Karunakaran, C., Tabil L., Schoenau, G., (2009). Potential applications of infrared and raman spectromicroscopy for agricultural biomass. *Agricultural Engineering International*, vol. XI.

Ali, M. M., Eisa ,M., Taha, M. I., Zakaria, B. A., and Elbashir, A. A. (2012). Determination of caffeine in some Sudanese beverages by high performance liquid chromatography. *Pakistan Journal of Nutrition*, 11 (4), 336-342.

Alpdogan, G., Karabina, K. and Sungur, S. (2002). Derivative spectrophotometric determination of caffeine in some beverages. *Turkish Journal of Chemistry*, 295-302.

Amos-Tautua, M., W., Bamidele and Diepreye, E.R.E. (2014). Ultraviolet spectrophotometric determination of caffeine in soft drinks available in Yenagoa, Nigeria. *Academic Journal of Food Science and Technology*, 6(2), 155-158.

Arya, M. and Rao, L.J.M. (2007). An impression of coffee carbohydrates. *Critical Reviews in Food Science and Nutrition*, 47, 51-67.

Ashu, R., and Chandravanshi, B.S. (2011). Concentration levels of metals in commercially available Ethiopian roasted coffee powders and their infusion. *Bulletin of Chemical Society of Ethiopia*, 25 (1), 11-24.

Atomssa, T., and Gholap, A.V. (2011). Characterization of caffeine and determination of caffeine in tea leaves using UV-Visible spectrophotometer. *African Journal of Pure and Applied Chemistry*, 5 (1), pp 1-8l.

Belay, A. (2011). Some biochemical compounds in coffee beans and methods developed for their analysis. *International Journal of the Physical Sciences,* 6 (28), pp 6373-6378.

Belitz, H. D., Grosch, W., and Schieberle, P. (2009). *Food Chemistry*. Berlin: Springer-Verlag.

Bernal, J.L., Nozal, M.J., Del, Toribio L., and Alamo, Del M. (1996). HPLC analysis of carbohydrates in wines and instant coffees using anion exchange chromatography coupled to pulsed amperometric detection. *Journal of Agriculture and Food Chemistry*, 44, 507-511.

Bianchi, F., Careri, M., Conti, M. and Vreuls, R. (2007). Comparison of comprehensive two dimensional gas chromatography time-of-flight mass spectrometry and gas chromatography-mass spectrometry for the qualitative characterization of roasted barley by solid-phase microextraction. *Journal of Separation Science,* 30, 527-533.

Briandet, R., Kemsley, E. K., and Wilson, R. H. (1996). Approaches to adulteration detection in instant coffees using infrared spectroscopy and chemometrics. *Journal of the Science of Food and Agriculture*, 359-366.

Butt, M.S., and Sultan, M. T. (2011). Coffee and its consumption. *Critical Reviews in Food Science and Nutrition*, 51, 363-373.

Careri, M., Bianchi, F., and Corrandini, C. (2002). Recent advances in application of mass spectrometry in food- related analysis. *Journal of Chromatography*, 970, 3-64.

Casal, S., Oliviera, B. P. P, Alves, M.R., and Ferreira, M.A. (2000). Discriminate analysis of roasted coffee varieties for trigonneline, nicotinic acid and caffeine content. *Journal of Agriculture and Food Chemistry,* 48, 3420-3424.

Cordella, C., Moussa, I., Martel, A., Sbirrazzuoli, N., and Lizzani-Cuvelier, L. (2002). Recent developments in food characterization and adulteration detection: technique oriented perspectives. *Journal of Agriculture and Food Chemistry,* 50, 1751-1764.

Cramer, J., Anne-chrystelle, M. D., Scott, F. J. K., and Baik, B. (2005). Analysis of volatile compounds from various types of cultivars. *Journal of Agriculture and Food Chemistry* , 53,pp. 7526-7531.

CSA, Central Statistics Agency (2012). Agricultural Sample Survey 2011/12 (2004 E.C) September-December 2011. Report on area and production of major crops (private peasant holdings Meher season). Vol 1, Addis Ababa. .

Dennis, M.J. (1998). Recent developments in food authentication. *Analyst*, 123, 151R–156R.

Domingues, D. S., Pauli, E., D., de Aberu, J. E.M., Massura, F. W., Cristiano, V.S., Maria, J., Nixdorf, S.L. (2014). Detection of roasted and ground coffee adulteration by HPLC by amperometric and by post-column derivatization UV-Vis detection. *Food Chemistry*, 146, 353-362.

Dong, L., Piao, Y., Zhang, X., Zhao, C. , Hou , Y. and Shi, Z. (2013). Analysis of volatile compounds from a malting process using headspace solid phase micro-extraction and GC-MS. *Food Research International*, 51, 783-789.

Dorea, J. G., and da Costa, T. H. (2005). Is coffee a functional food? *British Journal of Nutrition*, 93,773-782.

Ebrahimi-Najafabadi, H., Leardi, R., Oliveri, P. C., Maria, C., and Jalali-Heravi, L.S. (2012). Detection of addition of barley to coffee using near infrared spectroscopy and chemometric techniques. *Talanta* .

Elena, I., and Alejandro, C. (2001). New analytical techniques in food science. *Critical Reviews in Food Science and Nutrition*, 41 (6), 413.

Ferreira , S.L.C., Pereira, P.A.P, Nobrega, J.A., Fatibello-Filho, O., Feres, M.,A. Reis, B. F., Bruns, R.E., and Neto, F.R.A. (2008). A glimpse of Recent developments in Brazilian analytical chemistry. *Analytical Letters*, 41, 1494-1546.

Fontes, A.S., Bento , Baesso ,M.L., A.C and Miranda, L.C.M. (2006) .Thermal lens and PH measurements in pure and adulterated brewed coffee. *Instrumenation Science and Technology*.

George, S., E., Ramalakshmi., K., and Rao, L., J. M. (2008). A perception on health benefits of coffee. *Critical Reviews in Food Science and Nutrition*, 48, 464-484.

Gholizadeh, H., Nasserian, A., Valizadeh, R., Tahmasbi, A., and Yu P. (2014). Detecting chemical molecular structure difference among different Iranian barley cultivars using fourier transform infrared spectroscopy. *Annual Research and Review in Biology*, 4 (1), 258-268.

Gloess, A.N., Schonbachler, B., Klopprogge, B., D'Ambrosio, L., Chatelain, K., Bongartz, A. Strittmatter., A., Rast., M., and Yeretzian, C. (2013). Comparison of nine common coffee extraction methods: instrunmental and sensory analysis. *Eur Food Res Technol.*

Grover, I. S, Sharma, R. Singh., S., and Bonamali P. (2013). Polycyclic aromatic hydrocarbons in some grounded coffee brands. *Environmental Monitoring Assessment,* 185, 6459-6463.

Hayakama, F., Kazami, Y., Wakayama, H., Oboshi, R., Tanaka, H., Maeda Gou, H., Chiaki, I., Hidekazu, M. (2010). Sensory lexicon of brewed coffee for Japanese consumers, untrained coffee professionals and trained coffee tasters**.** *Journal of Sensory Studies*, 25, 917–939.

Heidelberg, B.M.T., Jimare, O.C.B. and Rojas, F.S. (2008). Process analytical chemistry :Applications of near infrared spectroscopy in environmental and food analysis: an overview. *Applied Spectroscopy Reviews,* 43, 452-484.

Higdon, J.V., and Frei, B. (2006). Coffee and Health: review of recent human research. *Critical Review in Food Science and Nutrition,* 46, 101-123.

Hillary, M., Swamy, T.A., and Kipngetich, T. (2013). Determination of caffeine and p^H levels of selected carbonated soft drinks and ready to drink juices in Eldoret, Kenya. *International Journal of Innovative Research in Engineering and Science,* 5 (2).

Houessou, J.K., Maloug, S., Leveque, A., Delteil, C., Heyd, B., and Camel, V. (2007). Effect of roasting conditions on the polycyclic aromatic Hydrocarbon content in ground Arabica coffee and coffee brew. *Journal of Agriculture and Food Chem*istry, 55, 9719-9726.

Hovell, A.M.C., Perira, E.J., Arruda, N.P., and Rezende, C.M. (2010). Evaluation of alignment methods and data pretreatment on the determination of the most important peaks for the discrimination of coffee varieties Arabica and Robusta using gas chromatography-mass spectroscopy. *Analytica Chimica Acta,* 678, 160-168.

Ibanez, E., and Cifuentes, A. (2001). New analytical techniques in food science. *Critical Reviews in Food Science and Nutrition*, 41(6), 413–450.

Jham, G. N., Winkler, J. K., Berhow, M.A., and Vaughn, S. F. (2007). Gamma tocopherol as a marker of Brazilian coffee (*Coffea Arabica* L.) adulteration by corn. *Journal of Agriculture and Food Chemistry,* 55, 5995-5999.

Kappes S.M., Schmidt, S.J., and Lee, S.Y. (2006). Mouth feel detection threshold of instrumental viscosity of sucrose and high fructose in corn syrup solutions. *Journal of Food Science*, 71 (9).

Kathurima, C.W., Gichimu, B. M., Kenji G.M., Muhoho S.M., and Boulanger R. (2009). Evaluation of beverage quality and green bean physical characteristics of selected Arabica coffee genotypes in Kenya . *African Journal of Food Science* , 3 (11), pp. 365-371.

Kemp, S.E., Hollywood, T., and Hort, J. (2009). *Sensory evaluation a practical handbook*. UK: Wiley-Blackwell.

Komes, D., Horzic, D., Belscak, A., Ganic, K. K., and Baljak, A. (2009). Determination of caffeine content in Tea and Mate tea by using different methods. *Czech Journal of Food Sci*ence, vol. 27.

Kukare A., Bartkevics, V., and Viksna A. (2010). Development of methodology for control of polycyclic aromatic hydrocarbon content in food. *Lativian Journal of Chemistry*, no 1-4, 54-65.

Kum Tatt, L. (1961). A routine method for determining caffeine in coffee and coffee mixture. *Analyst*, 86, 825.

Lau, O., Luk, S., Cheng, O., and Chiu, T.P.Y. (1992). Background correction methods for the determination of caffeine in beverages, coffee and tea by using second-derivative ultraviolet spectrophotometry. *Analyst*, vol. 117.

Lee, K., and Shin, H. (2010). Determintion of polycyclic aromatic hydrocrbons in commercial roasted coffee beans. *Food Science and Biotechnology*, 19(6), 1435-1440.

Moraes, P.C.B.T. and Bolini , H.M.A. (2010). Different sweeteners in beverages prepared with instant and roasted ground coffee: ideal and equivalent sweetness .*Journal of Sensory Studies*, 25,215-225.

Murray, J.M., Delahunty, C.M., and Baxter, I.A. (2001). Descriptive sensory analysis: past present and future. *Food Research International*, 34, 461-471.

Nogueira, T., and do Lago, C. L. (2009). Detection of adulteration in processed coffee with cereals and coffee husks using capillary zone electrophoresis. *Journal of Separation Science*, 32, 3507-3511.

Nolan, L. (2001). The world's favorite beverage coffee and health. *Journal of herbs, spices & medicinal plants,* Vol.8 (2/3)

Oestreich-Janzen, S., (2010). *Chemistry of Coffee*. CAFFEA GmbH. Germany: Elsevier.

Oliveira, A. L., Cabral, F.A., Eberlin, M. N., and Cordello, H. M. A. (2009). Sensory evaluation of black instant coffee beverage with some volatile compounds present in aromatic oil from roasted coffee. *Ciencia e. Technologia de Alimentos Campinas*, 29 (1), 76-80.

Oliveira, A., L., Cruz, P. M., Eberlin, M. N., Cabral, F.A. (2005). Brazilian roasted coffee oil obtained by mechanical expelling: compositional analysis by GC-MS. *Ciencia e. Technologia de Alimentos Campinas*, 25 (4),677-682.

Oliveira, R..C.S., Oliveira, L.S., Franca, A.S., and Augusti, R. (2009). Evaluation of the potential of SPME-GC-MS and chemometrics to detect adulteration of ground roasted coffee with roasted barley. *Journal of Food Composition and Analysis*, 22, 257-261.

Olmos, V., Bardoni, N., Ridolfi, A.S., and Villaamil Leopri , E. C. (2009). Caffeine levels in beverages from Argentina's market: application of caffeine dietary intake assessment. *Food Additives and Contaminants*, 26 (3), 275-281.

Orecchio, S., Ciotti, V.P., and Culotta, L. (2009). Polycyclic aromatic hydrocarbons (PAHs) in coffee brew samples .Analytical method by GC-MS, Profile, levels and sources. *Food and Chemical Toxicology,* 47, 819-826.

Petit, N. (2007). Ethiopia’s coffee sector: a bitter or better future. *Journal of Agrarian Change*, Vol. 7 No. 2, pp. 225–263.

Pena, A., Lino, C., and Silveira, M.I.N. (2005). Survey of caffeine levels in retail beverages in Portugal. *Food Additives and Contaminants*, 22 (2), 91-96.

Penalver, A., Pocurull, E., Borrull, F., Marce, R.M. (2000). Determination of phthalate esters in water samples by solid-phase micro extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography* A, 872, 191-201.

Prakash M., Ravi, R., Sarvamangala, G.K., and Rajalakshmi, D. (1998). Sensory profiling and poduct positioning of roasted and ground (brew) coffee with and without added flavor. *Journal of Sensory Studies*, 15, 101-117.

Prodolliet, J., and Hischenhuber, C. (1998). Food authentication by carbohydrate chromatography. *Z lebensm unters forsch A.*, 207, 1-12.

Pujol, D., Liu C., Gominho, J., Olivella , M.A., Fiol, N., Villaescusa, I., and Pereira, H. (2013). The chemical composition of exahust coffee waste. *Industrial and Crop Products*, 50, 423-429.

Raghavan, B. and Ramalakshmi , K. (1999). Caffeine in coffee: its removal. Why and how? *Critical Reviews in Food Science and Nutrition*, 39(5), 441-456.

Reis, N., Franca., A.S., and Oliveira, L.S., (2013). Performance of diffuse reflectance infrared fourier transform spectroscopy and chemometrics for detection of multiple adulterants in roasted and ground coffee. *Food Science and Technology*, 53, 395-401.

Ribeiro, J.S., Ferreira, M.M.C., and Salva, T.J.G. (2011). Chemometric models for quantitative descriptive sensory analysis of Arabica coffee beverages using near infra red spectroscopy. *Talanta*, 83, 1352-1358.

Ridgeway, K., Lalljie, S.P.D., and Smith R.M. (2009). Analysis of food taints and off-flavors: a review. *Food Additives and Contaminants*, 27 (2), 146-168.

Rojo, C. M. C., Toledo, M., Celcilla, F., and Farah, H. G. (1999). Caffeine daily intake from dietary sources in Brazil. *Food Additives and Contaminants*, 16 (2), 79 -87.

Rudolph, E., Farbinger, A., and Konig J.,(2012). Determination of the caffeine content of various food items within the Austrian market and validation of a caffeine assessment tool (CAT).*Food Additives and Contaminants* A, 29 (12), 1849-1860.

Sadowska-Rockie, A., Surma M., and Cieslik E. (2015). Determination of polycyclic aromatic hydrocarbons in coffee and coffee substitutes using Dispersive SPE and Gas Chromatography-Mass Spectrometry. *Food Analytical Methods*, 8, 109–121.

Sano, E.E., Assad, E. D., Cunha, S.A.R,. Correa, T.B.S., and Rodrigues, H.R., (2003). Quantifying adulteration in roast coffee powders by digital image processing. *Journal of Food Quality*.

Santos, K. M., Moura, M.F.V., Azevedo, F.G., Lima, K.M.G., Raimundo, I. M., and Pasquini, C. (2012). Classification of Brazilian coffee using near-infrared spectroscopy and multivariate calibration. *Analytical Letters*, 45,774-781.

Sanz, C., Maeztu, L., Zapelena, M., Bello, J., and Cid, C. (2002). Profiles of volatile compounds and sensory analysis of three blends of coffee: influence of different proportions of Arabica and Robusta and influence of roasting coffee with sugar. *Journal of the Science of Food and Agriculture*, 82, 840-847.

Sarraguca, M. C., Santos, J.R., Rangel, A.O.S.S., and Lopes, J.A. (2013). Authenticity control of roasted coffee brands using near infrared spectroscopy (2013). *Food Analytical Methods*, 6, 892-899.

Seo, H., Lee, M., Jung Young, J., and Hawang, I. (2009). A novel method of descriptive analysis on hot brewed coffee: time scanning descriptive analysis. *Eur Food Res Technol*, 228, 931-938.

Seo, H., Lee, S., and Hwang, I. (2009). Development of sensory attribute pool of brewed coffee. *Journal of Sensory Studies*, 24,111-132.

Shewayrga, H., and Sopade, A. P. (2011). Ethno botany, diverse food uses, claimed health benefits and implications on conservation of barley landraces in North Eastern Ethiopia highlands. *Journal of Ethno Biology and Ethno Medicine,* 7, 19.

Smith, A. (2002). Effects of caffeine on human behavior . *Food and Chemical Toxicology*, 40, 1243-1255.

Smith R.F., and Rees, D.I. (1963). The spectroscopic determination of caffeine in coffee and tea products with special reference to coffee and chicory mixture. *Analyst*, vol. 88.

Stone, H., and Sidel J.L. (1993). Sensory evaluation practices, 2nd (Ed.), Orland Academic press.

Souto U. T. D., Polari, Barbosa, M.F., Dantas H. V., de Pontes, A. S., Lyra, W.,D., D., Paulo. H. G. D., de Araujo, M. C. U. (2015). Identification of adulteration in ground roasted coffees using UV-Vis spectroscopy and SPA-LDA. *Food Science and Technology*,1-5.

Suteerapataranon, S., Jurairat, B., Pantiwa, P.W., and Punturat, C.T. (2009). Caffeine in chiang rai tea infusion: effect of tea variety, type, leaf form and infusion condition. *Food Chemistry*, 114, 1335-1338.

Sualeh, A., and Mekonnen, N. (2013). Evaluation of coffee quality of Tigray regional state, Ethiopia (Case study of Raya Azebo district). *Prudence Journal of Agricultural Research*, 1 (2), pp 3-6.

Sualeh, A., Endris, S., and Ali, M. (2014). Processing method, variety and roasting effect on cup quality of Arabica coffee (*Coffea Arabica). Discourse Journal of Agriculture and Food Sciences,* 2(2), 70-75.

Tfouni ,S.A.V. Serrate,C.S., Carreiro L.B., Camargo ,M.C.R., Teles, C.R.A., Cipolli, K. M.V.A.B., and Furlani R.P.Z.,(2012). Effects of roasting on chlorogenic acids, caffeine and polycyclic aromatic hydrocarbons levels in two Coffea cultivars: coffea Arabica cv. catuai Amarelo IAC-62 and coffea canephor acv. Apoata IAC-2258. *International Journal of Food Science and Technology* , 47, 406–415.

Tzouros, N.E., and Arvanitoyannis I.S., (2001). Agricultural produces: synopsis of employed quality control methods for the authentication of foods and application of chemometrics for the classification of foods according to their variety or geographical origin. *Critical Reviews in Food Science and Nutrition* , 41(4), 287-319.

QU, J., Liu, D., Cheng , J., Da-Wen, S., ,Ji, M. , Pu , H., and Zeng , X. (2014). Application of near infra red spectroscopy in food safety evaluation and control: a review of recent advances. *Critical Reviews in Food Science and Nutrition*.

Varvolgyi, E., Werum, T., Denes, L.D, Kovacs, Z., Szabo, G., Felfoldi, J., and Esper , G. (2013). Comparison of the discrimination power of the electronic tongue, near infra red spectroscopy and sensory analysis regarding the adulterant Barley in Robusta coffee. *In proceedings of food, technology and health. International scientific-practical conference*. Food Research Development Institute: Agricultural Academy.

Violeta, N., I, Trandafir, and Elena, I.M. (2008). Quantitative determination of caffeine in carbonated beverages by an HPLC method. *Journal of Agroalimentary Processes and Technologies*, 14, 123-127.

Wang,Y., and Ho, C. (2009). Polyphenolic chemistry of tea and coffee: a century of progress. *Journal of Agricultural and Food Chemistry,* 57, 8109-8114.

Wang, P., Kato, H., and Fujimaki, M. (1969). Studies on flavor components of roasted barley. Part III. The major volatile basic compounds. *Agriculture and Biological Chemistry*., 33 (12), pp.1775-1781.

Wang, N., and Lim, L. (2012). Fourier Transform infrared and physicochemical analysis of roasted coffee. *Journal of Agriculture and Food Chemistry*, 60, 5446−5453.

Wanyika, H.N., Gatebe, E.G., Gitu, L.M., Ngumba, E.K. and Maritim, C.W. (2010) Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. *African Journal of Food Science,* 4 (6), pp 353-358.

Welna, M., Szymczycha-Madeja, A., and Zirnicki, W. (2013). Applicability of ICP-OES, UV-VIS and FT-IR methods for the analysis of coffee products. *Analytical Letters*, 46, 2927-2940.

Worako, T.K., Van Schalkwyk, H.D., Alemu, Z.G., and Ayele, G. (2008). Producer price and price transmission in a deregulated Ethiopian coffee market. *Agrekon*, 47 (4).

Zhou, M., Robards, K., Glennie-Holmes, M. and Helliwell, S. (1999). Analysis of volatile compound and their contribution to flavor in cereals. *Journal of Agriculture and Food Chemistry*, 17 (10).