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PROFILING THE FATTY ACID CONSTITUENTS IN GREEN COFFEE BEANS FROM SOME SELECTED AREAS OF AMHARA REGION

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June, 2018

Bahir Dar, Ethiopia

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Thesis

Submitted to the college of science post graduate program in partial fulfillment to the requirements for the degree of Master of Science in analytical chemistry

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The thesis titled "**PROFILING THE FATTY ACID CONSTITUENTS IN GREEN COFFEE BEANS FROM SOME SELECTED AREAS OF AMHARA REGION"** by Tibebu Shiferaw is approved for degree of Master of Sciences in Analytical Chemistry

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DECLARATION

This is to certify that thesis entitled "PROFILING THE FATTY ACID CONSTITUENTS IN GREEN COFFEE BEANS FROM SOME SELECTED AREAS OF AMHARA REGION" submitted in partial fulfillment of the requirements of the degree of Master of Science in Analytical Chemistry to the post Graduate Program of College of Science, Bahir Dar University is an authentic work conducted by Tibebu Shiferaw Kassa under the supervision of Minaleshewa Atlabachew (PhD)

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ABBREVIATION

ANOVA	One-why Analysis of Variance
AOAC	Association of Official Agricultural Chemist
AZARDO	Awi Zone Agricultural and Rural Development Office
EGZARDO	East Gojjam Zone Agricultural and Rural Development Office
ECEA	East Africa Fine Coffee Association
EV	Electron-Volt
FAO	Food and Agriculture Organization
ICO	International Coffee Organization
ITC	International Trade Center
MOA	Ministry of Agriculture
QSAE	Quality and Standards Authority of Ethiopia
RPM	Revolution Per Minute
STD	Standard Deviation
WGZARDO	West Gojjam Zone Agricultural and Rural Development Office

ABSTRACT

Coffee Arabica is highly cultivated in different areas of Amhara region, specifically East Gojjam, West Gojjam, and Awi zones. However, this coffee has not been well characterized and branded for the national and international market. Therefore, this study was aimed to profile the fatty acid contents of green coffee beans from West Gojjam, Awi, and East Gojjam zones of the Amhara region. Twenty four samples of green coffee (Coffea Arabica) beans collected from the major producing zones, comprising various Woredas, were studied for variations in their fatty acid compositions. The lipid fractions of the powdered green coffee beans were extracted using solvent extraction method and the fatty acid portion of the lipid fraction was derivatized to the corresponding fatty acid methyl esters (FAMEs). The individual fatty acids were identified and quantified using gas chromatography coupled with mass spectrometry (GC-MS) following standard procedures. Data were analyzed with SPSS version 22.0. Twenty three fatty acid types were detected: Among these myristic (C14:0), pentadecanoic acid (C15:0), palmitic (C16:0), margaric acid (C17:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), arachidic (C20:0), gondoic acid (C20:1),14-methyleicosanoic acid (C21:0), behenic acid (C22:0), tricosanoic acid (C23:0) and lignoceric acid (C24:0) were the major ones. There was a significant difference ($p<0.05$) between zones and woredas in the concentration of total fatty acids. The total fatty acid contents of West Gojjam (102. 74 mg g^{-1}) significantly higher than from East Gojjam and Awi zones were 84.56 and 90.55 mg g^{-1}respectively and Bure city administration coffees samples from West Gojjam zone contained (123.54 mg g^{-1}) of the total fatty acid content significantly higher than the rest woredas coffee types ranged from (78.45−104.88 mg g^{-1}). Myristric, *palmitic, linoleic, stearic, arachidic and trieicosanoic acid were identified as the most important compounds for discrimination of coffee samples based on their geographical origin. The studied fatty acids provide adequate information for use as descriptors of the geographical origin of coffee beans from Amhara region.*

Keywords: Green Coffee Beans; Fatty acids; Geographical origin.

1. INTRODUCTION

1.1. Background and Justification

Coffee is one of the most popular drinks in the world. From the commercial point of view, only Coffee Arabica and Coffee Robusta represent the two most relevant and widely cultivated species in the world (Delgado *et al*., 2008), and also the most commercially available coffee mixtures are in fact obtained from Arabica and Robusta blends. These typologies differ not only in relation to their botanical, chemical, and organoleptic characteristics but also in terms of commercial value; in fact, coffee Arabica is more expensive due to their high quality (Reglero *et al*., 2003). Green coffee beans of the Arabica and Robusta varieties can be distinguished by their size, shape, and color, but the roasting process eliminates these macroscopic aspects (Araujo and Sandi, 2007).

Coffea Arabica Commonly known as Arabica coffee is the coffee species most appreciated by consumers. It represents around 70% of the total world coffee production and provides a high-quality brew compared to Coffee Robusta due to its intense aroma, low bitterness, and low caffeine content (Lashermes and Anthony, 2007). It is one of the most agricultural products consumed in the world, from which a popular beverage is prepared for its particular aroma, taste, and composition (Delago *et al*., 2008).

Ethiopian coffee is an important source of coffee genetic resources for the world coffee industry. According to Anthony *et al*., (2001) reports, Ethiopia is the only center of origin and diversity of Arabica coffee (*C. Arabica*). It is cultivated in most parts of the tropics, accounting for 4.4 percent of the world coffee market (Tadesse *et al.,* 2002). It is also an important source of income and employment in developing countries of Latin America, Africa and Asia (Anthony *et al.,* 2001).

Several researches have been made to develop an analytical method to establish the source of green coffee beans at different countries. Bertrand *et al.,*(2008) investigated that the possibility of using fatty acid composition as determined by gas chromatography (GC), elemental content as determined by inductively coupled plasma optical emission spectrometry *(*ICP-OES*),* and chlorogenic acid profiles determined by high-performance liquid chromatography (HPLC), for distinguishing from Colombian coffees. Regarding Ethiopian coffees, there are a number of studies that have compared the phenolic

methylxanthine (Alonso-Salces *et al*., 2009), elemental and stable isotope (Rodrigues *et al.,* 2009; Lilu *et al.*,2014) compositions of some of the country's coffee types with samples from other countries. Ultra-performance liquid chromatography hyphenated with quadrupole time-of-flight mass spectrometry (UPLCqTOF-MS) was used for profiling phenolic compounds in green coffee beans from Ethiopia (Mehari *et al*., 2016).

As Alonso-Salces *et al.,* (2009) mentioned that green coffee bean has the largest lipid fraction occurs in the oil present in the endosperm of the bean. This oil consists of triglycerides, phospholipids, sterols, and diterpenes. Among these compounds are mainly present as esters of fatty acids. Lipids in coffee serve as carriers of flavors and fat-soluble vitamins and contribute significantly to the organoleptic qualities of the beverage, such as texture and mouthfeel. Fatty acids constitute about 75% of the lipids in the green beans. Arabica is considered superior to Robusta in its beverage quality. The lipid composition of green coffee beans is regarded as an important parameter to distinguish between the two as well as geographical origin varieties. Despite the importance of compound class in coffee, no information is available on the lipid and fat compositions of in green coffee beans of West Gojjam, East Gojjam, and Awi zonse from Amhara region.

The fatty acid profiles of green coffee mainly depend on the variety of the coffee, although slight variations are possible due on genetic traits, agro-economic practices, and harvesting, post-harvest conditions and environmental factors (Villareal *et al.*, 2009; Joet *et al.,* 2010). Since these factors may differ from one area to another, the concentrations of fatty acids can be used for geographical origin determination of green beans coffee. Fatty acids are one part of lipids which differ in chain length, in the degree of saturation, configuration, the position of the double bonds, and the presence of other functionalities (Gutnikov, 1995). Studies carried out in obtaining oil from coffee with different extraction methods, reported the presence of saturated and unsaturated fatty acids, mainly palmitic acid, stearic, oleic, linoleic and arachidic acid (Ramirez, 2008).

The aim of this study is profiling the fatty acid constituents of green coffee beans from West Gojjam, Awi and East Gojjam zones in Amhara region, was achieved based on an analytical method for the fast extraction of fat from coffee beans with simultaneous derivatisation to FAMEs and independent identification and quantification of fatty acids using GC–MS.

1.2. Statement of the problem

Green coffee is composed primarily of water, carbohydrates, fiber, proteins, free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline, and caffeine. Of these compounds found in green coffee beans, chlorogenic acids, caffeine, trigonelline,, soluble fiber, and diterpenes from the lipid fraction are most likely to be bioactive, and they may also be important contributors to the beverage flavor after roasting (K¨olling-Speer and Speer, 2005).

The lipid fraction of coffee Arabica is composed mainly of tri-glycerol (75%), fatty acids (1%), sterols (2.2%), unesterified and esterified with fatty acids (3.2%) and tocopherols (0.05%), which are typically found in edible vegetable oils and Fatty acids in coffee are found primarily in combined forms; most are esterified with glycerol in the tri-glycerol fraction, 20% esterified with diterpenes, and a small proportion in sterol esters (Kyng *et al*., 1999).

Several reports have indicated that the fatty acid composition in green coffee beans is one of the positional marker substances to characterize the geographical origin of coffee at regional, sub-regional and continental level (Martín *et al*., 2001). Among the chemical composition, lipids and fats are higher in Arabica coffee (Feldman *et al*., 1969). Even though Coffee Arabica is highly cultivated in different areas of Amhara region, specifically, East Gojjam, West Gojjam, and Awi zones; there is no scientific research conducted in Ethiopia. Hence, it is a pressing demand for characterizing the fatty acid constituents of the coffee region for branding.

1.3. Objectives

1.3.1. General objective

The overall objective of this study was profiling the fatty acid constituents in green coffee beans from some selected areas of Amhara region using GC-MS.

1.3.2. Specific objectives

- ✓ To identify and quantify the fatty acid composition of green coffee beans of West Gojjam, East Gojjam, and Awi zones.
- ✓ To identify saturated and unsaturated fatty acids in different coffee bean samples.

- ✓ To identify the major dominant fatty acid in West Gojjam, East Gojjam, and Awi zones green coffee beans.
- ✓ To compare the fatty acid constituents of green coffee beans in three zones with each other and with reported values.

1.4. Research Question

Is the fatty acid profile of East Gojjam, West Gojjam, and Awi zone coffee different with each other or not?

Is it possible to get enough but distinct fatty acid profile to discriminate coffee of East Gojjam, West Gojjam, and Awi zone with each other or not?

Which fatty acid is more responsible to characterize the various coffee beans grown in the three zones?

1.5. Significance of the Study

Identification and Quantification of the fatty acid constituents of green coffee beans of East Gojjam, West Gojjam, and Awi zones may be used to find marker element which can characterize a particular coffee for branding. The output of this would provide empirical support for the continued effort for branding the coffee that is produced in the East Gojjam, West Gojjam, and Awi zones like that of other well known coffee producing regions of Ethiopia and may also be used for traceability of coffee's geographical origin.

2. LITERATURE REVIEW

2.1. Coffee Arabica

Coffee *(Coffea Arabica L.*) is a non-alcoholic stimulant beverage crop that belongs to the family *Rubiaceae* and genus *Coffea.* Arabica coffee is an evergreen shrub of variable size (**Figure 1**). The tree grows up to 8-10 m high and its branches are long, flexible and thin. Branches are semi-erect when young and spreading or pendulous when old (Coste, 1992). The architecture of the coffee tree is characteristic of a tree growing in tropical forests; a vertical (orthotropic) stem, with horizontal (plagiotropic) branches arising in pairs opposite to each other. The growth is by a typical form of monopodial branching where the branches (primaries) remain subsidiary to the main stem, which continues to grow indefinitely by extension of the apical buds (Wrigley, 1988). The coffee plant takes approximately three years to develop from seed germination to first flowering and fruit production. A well-managed coffee tree can be productive for up to 80 years or more, but the economic lifespan of a coffee plantation is rarely more than 30 years (Wintgens, 2004).

The size and shape of the beans differ depending upon the variety, environmental conditions, and management practices. On average, beans are 10 mm long, 6-7 mm wide, 3-4 mm thick and weigh between 0.15 and 0.20 g. Bean color can be yellowish-grey to slate-grey, bluish or grey-green, depending upon the variety, method of preparation and storage condition (Coste, 1992). Bean shape may be sub-globular, ovoid, oblong, linear-oblong, either rounded at both ends or pointed at one end and rounded at the other (FAO, 1968).

There are two varieties of the coffee plant with economic importance; Coffea Arabica and Coffea Canephora, known in the trade as Arabica and Robusta Respectively. Coffee beverages are made from roasted beans belonging to one of these two varieties or blends of them. The better quality coffees, and thus the most expensive ones, are considered to be the Arabicas. Frequently, green coffee beans of the Arabica and Robusta varieties can be distinguished by their size but the roasting process eliminates this macroscopic criterion. Therefore, reliable methods are required to differentiate these varieties (Briandet *et al*., 1996).

a)  b)  c)  d) 

Figure 1: Image of the coffee plant (**a &b**), coffee cherries(**c**) and green coffee beans (**d**) (Department of Agriculture, Forestry, and Fisheries, 2012).

2.2. World Coffee Production Marketing Overview

2.2.1. World coffee production

Coffee, together with tea, is one of the most popular drinks in the world. It is the second most traded commodity in the world after oil (Delgado *et al*., 2008). The first coffee plantations were originally established in Ethiopia and the Arabian Peninsula and it was introduced to Asia and, later, to Latin America by the Dutch, who became the main suppliers of coffee to Europe in the 18th century. Today it is widely grown throughout tropical regions (ITC, 2008). Most of the world's green coffee beans are produced mainly in South America, Brazil is the first producing country (42%) since 1840. Africa accounts for 20.4% of the total production and Asia produces 18.5%. Nevertheless, Europe is the main coffee consumer (Smith *et al.*, 1985). In 2006 more than half of the global coffee production was concentrated in three countries: Brazil, Vietnam, and Colombia (Roldan-Pérez, 2007).

Global coffee production averaged around 6 million tons a year during the 1990s. Increased output from Brazil and Vietnam production grow to an average of 7.6 million tons a year between 2007 and 2011, peaking at a record 8.05 million tons in 2010 (Jaffee, 2012).

Brazil is the world's largest coffee producer and exporter. Vietnam expanded its production rapidly throughout the 1990s and now holds the number two position, displacing Colombia into third place and Indonesia into fourth. In 1976, eight countries shared 60 percent of world coffee production (Brazil, Colombia, Cote d'Ivoire, Ethiopia, Indonesia, Mexico, Uganda and El Salvador) but with the rise of Vietnam as the second largest coffee producer in 1999, just

four countries (Colombia, Brazil, Vietnam and Indonesia) produced 60 percent of the world's coffee (Roldan-Pérez 2007).

2.2.2. Coffee consumption in the world

According to the International Trade Centre (2008), global consumption in coffee year 2006/07 totaled 125.9 million bags. That constituted a 2.26 percent growth on the previous year (89.85 million bags in 2005/2006) (ICO 2008)). In 2006/2007, importing countries' consumption accounted for 73 percent of global consumption (91.8 million bags) with an average annual growth since 2002/2003 of 8.72 percent. Conversely, coffee consumption in producing countries for the same year accounted for 27 percent of global demand (34.02 million bags)). In 2008, world coffee consumption is estimated to have reached 128 million bags, comprising 79 million bags of Arabica and 49 million bags of Robusta (ICO, 2009a). According to an International coffee organization (2017/18), global consumption in coffee year 2012/13-2015/16 in the different country is listed below in **Table 1.**

Table 1: world coffee consumption in thousand 60 kg bags. (Source: (ICO, 2017/18)

Global consumption of coffee					
World	2012/13	2013/14	2014/15	2015/16	2012-2015/16
Total	146 964	149 022	151 758	155 469	1.9%
Africa	10470	10 597	10 754	10 794	1.0%
Asia & Oceania	29445	30 701	32 550	33 611	4.5%
C/America & Mexico	5 200	5 156	5 235	5 306	0.7%
Europe	50028	50 179	50 912	51 590	1.0%
N/America	26778	27 706	27 359	28 931	2.6%
S/ America	25042	24 682	24 949	25 237	0.3%

2.2.3. Coffee producing countries

The International Coffee Organization (ICO) has divided world coffee production into four groups based on the prevalent type of coffee produced by each member country. However, many countries produce both Arabica and Robusta (**ICO, 2009a).**

Table 2: Coffee producing countries

Quality Group	Producers
Colombia and mild Arabica	Colombia*, Kenya, United Republic of Tanzania
Other mild Arabica	Bolivia, Burundi, Cameroon, Congo Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, United States,Guatemala,Haiti,Honduras,India, Indonesia, America, Madagascar, Malawi, Mexico*,Nicaragua, Nigeria, Panama, Papua New Guinea, Peru, (Puerto Rico),Rwanda, Venezuela, Zambia, Zimbabwe
Brazilian and other natural Arabica	Brazil*, Ethiopia, Paraguay
Robusta	Angola, Benin, Brazil, Cameroon, Central African Republic, Côted'Ivoire, Democratic Republic of the Congo, Ecuador, Equatorial Guinea, Gabon, Ghana, Guinea, India, Indonesia, Liberia, Malaysia, Madagascar, Nigeria, Philippines, Sierra Leone,Sri Lanka, Thailand, Togo, Trinidad and Tobago, Uganda, Vietnam

2.2.4. Coffee production and export in East Africa

The coffee plant is indigenous to Africa, and it was in Ethiopia that the habit of drinking coffee first developed. The two botanical varieties, Arabica and Robusta, originate from Africa. Robusta coffee is cultivated at lower altitudes while Arabica coffee is cultivated at higher altitudes and often on volcanic soils. Arabica coffee is more difficult and costly to grow than Robusta. Coffee is also the primary source of income for more than 10 million households in 25 African coffee-growing countries. Some of these countries depend on coffee as an important source of export revenues. It is a vital contributor to foreign exchange earnings in addition to accounting for a significant proportion of tax income and Gross Domestic Product (Arfaoui *et al.*, 2016).

As of International Coffee Organization (ICO, 2015), of Africa is the region with the largest number of coffee producing countries: 25 as opposed to 11 in Asia & Oceania, 12 in Mexico & Central America and 8 in South America. Production in Africa has exhibited negative

growth over the last 49 years. Average production was 19.4 million bags per crop year in the period between 1965/66 and 1988/89 when the coffee market was regulated under the export quota system. During the period between 1989/90 and 2014/15 under the free market, average production per crop year was 16 million bags. During those two periods, Africa's share of world production has hence decreased from 24.9% to an average of 14%. Production in crop year 2014/15 was around 16.9 million bags or 12% of the estimated world production of 141.7 million bags.

2.2.5. Taxonomic classification and species of Ethiopian coffee

Coffee belongs to the genus *Coffea*, in the *Rubiaceae* family Coste, 1992). The family *Rubiaceae* consists of some 500 genera (ITC, 2002), of which *Coffea* is economically the most important (Wellman, 1961). Based on the literature, herbarium specimens and wide field experiences, Chevalier (1947) classified the genus *Coffea* into four sections**:** *Eucoffea* K Schum, *Argocoffea* Pierre, *Mascarocoffea* Chev and *Paracoffea* Miq. The first three sections of the genus *Coffea* are exclusively native to Africa, Madagascar, and some adjacent Islands.

On the other hand, most representatives of *Paracoffea* are indigenous to India, Malaysia, Ceylon and Southeast Asia. Cultivated species of the genus *Coffea* belongs to the section *Eucoffea. Eucoffea* is divided into five subsections, fruit color (*Erythrocoffea, Melanocoffea),* tree height *(Nanocoffea),* and leaf thickness (Pachycoffea and geographical distribution (Mozambicoffea) (FAO, 1968b). Although the genus *Coffea* comprises about 100 species, of all the species, only two *Coffea Arabica* and *Coffea Canephora* have commercial value in the world coffee industry. *Coffea Arabica* is the only species occurring in Ethiopia and is geographically isolated from the rest of the *Coffea* species (Pearl *et al*., 2004).

2.2.6. Arabica coffee and its production systems in Ethiopia

Coffea Arabica is indigenous to Ethiopia and the principal source of foreign currency. It is mainly produced in the Southern, South Western and Eastern parts of the country. Forests in Southwestern of Ethiopia are the primary center of origin and center of genetic diversity of *Coffea Arabica* (Melaku, 1982).

Ethiopia is the main coffee producing country in Africa and the fifth worldwide (I CO, 2013)**.** Coffee accounts for 24% of Ethiopia’s foreign exchange earnings (Minten *et al.*, 2014) and

contributes to the livelihood for more than a quarter of the country's population (Tefera, 2014). Over the period 1990 to 2013, coffee production increased from 2.9 million bags (with one bag equivalent to 60 kg) to 8.1 million bags; and exports increased from 0.85 to 3.2 million bags (ICO, 2013). About 95% of coffee production is realized by smallholder farmers with average landholdings below 2 ha and sometimes organized in cooperatives (Francom and Tefera, 2016).

Ethiopia is endowed with a good production environment for growing coffee with a combination of appropriate altitude, temperature, rainfall, soil type, and PH. Ethiopia is the center of origin for *Coffea Arabica*. The country possesses a diverse genetic base for this Arabica coffee with considerable heterogeneity. It is mainly produced in the regions of Oromia and Southern Nations, Nationalities and People's Republic (SNNPR) (Petit, 1987).

Coffee is produced under four different production systems, along with an intensification gradient: forest coffee accounts for 10% of total coffee production; semi-forest coffee accounting for 35%; garden coffee for 50%; and plantation coffee for 5% (Kufa, 2012).

Forest Coffee: Forest coffee is self-sown and grows under the heavy shade of natural forest trees in the South and Southwest Ethiopia (Bale, West Wollega, Bench-Maji, Gamo Gofa, Keficho Shekicho, Metu, and Jammer areas). Forest coffee offers a wide diversity of selection and breeding for disease resistance, high yields, and top quality in terms of aroma as well as flavor. Forest coffee accounts for about 10% of the total coffee production (Tesfu, 2012).

Semi-Forest Coffee: Farmers thin and select forest trees to let in adequate sunlight to the coffee trees while still providing adequate shade. Farmers slash the weeds once a year to facilitate harvesting of the coffee beans. This production system is predominant in the south and southwest parts of the Country. This system accounts for about 35% of the coffee production (Tesfu, 2012).

Garden Coffee: Smallholder farmers grow garden coffee near their residences mainly in the southern and eastern parts of the country (Sidamo, Gedeo, South and North Omo, Hararghe, Wollega, and Gurage Zones). This coffee plant at densities ranging from 1000 to 1800 trees per hectare, mostly fertilizes with organic material, and intercrop with various crops. This coffee plantation system accounts for about 50% of the total production (Woldemariam *et al*., 2014).

Plantation Coffee: The state and some private investors grow large-scale plantation coffees with sufficient technological inputs, most of which are located in the Southwest. In this production System, farmer use recommended agro-economic practices like raising seedlings, mulching, manuring, weeding, shade regulation, and pruning on a regular basis. Plantation coffee accounts for about 5% of the total production (Tesfu, 2012).

Overall coffee growing districts in Ethiopia are classified as major, medium, and minor coffee growers based on the area covered by coffee trees and they are concentrated mainly in the Oromia Regional State and the Southern Nations, Nationalities and Peoples' Region (SNNPR). Major and medium growing Woredas contain an estimated 800,000 coffee farmers with approximately 520,000 ha under coffee, of which 63.3 percent is in the Oromia region, 35.9 percent is in SNPP region and 0.8 percent is in the Gambella region (MoA, 2003). Other non-traditional regions of the country also are experiencing coffee production.

2.2.7. Coffee productions in Amhara region

The most productive areas of coffee in Ethiopia are located in SNNPE and Oromia region but Amhara region is also good production area of coffee bean. From the most coffee producing areas of in Amhara region are; Eastern Gojjam, Western Gojjam, and Awie zone, Southern Gondar, Northern Shoa and Wollo produce different types of coffee with different flavor and stimulation. Furthermore, natural forests found in various monasteries of the region are known to be home of a genetic diversity of Arabica coffee (Alemayehu *et al*., 2008).

However, From the early 19^{th} to the last decade of 19^{th} centurycoffee from Zegie peninsula coffee had become a dominant cash crop in Zegie peninsula and was exported into British Sudan through Mettema, a town on the Ethio-Sudanese border. In the first three and a half decades of the 20^{th} century, its lucrative revenue kindled both local and international merchants (Bahiru, 2002).

2.3.Quality of Green Coffee Arabica

Coffee beans quality depends on moisture content, defects, bean size, some chemical compounds and preparation of a sample to perform cup tasting. Physical analysis to characterize the technological quality of green coffee is also used due to its promptness and easiness, as well as to its lower costs. In this context, color has been used for quality

assessment of green coffee beans and there have also been studies using image processing for grain quality inspection (Leroy *et al*., 2006).

Quality assessment of coffee genotypes at a chemical level can also be used, namely for identification of chemical clusters discriminators of the roast degree in coffee beans, identification of nutritional descriptors of roasting intensity in beverages of coffee beans, identification of chemical clusters discriminators of Arabica and Robusta green coffee and impact of roasting time on the sensory profile of Arabica and Robusta coffee (Woldetsadik, 2000).

Coffee is the most important crop in the national economy of Ethiopia and the leading export commodity. Ethiopia is well known not only for being the home of Arabica coffee but also for it is very fine quality coffee acclaimed for its aroma and flavor characteristics. The coffee types that are distinguished for such unique characteristics include Sidamo, Yirga Chefe, Harerge, Gimbi and Limu types (Woldetsadik, 2000). However, the coffee produced in some parts of Ethiopia, especially from Harrar, and Yirgachefe, is always sold at a premium price both at domestic and international coffee markets because of its distinctive fine quality (ITC, 2002) and appropriate processing approach.

According to the International Organization for Standardization (ISO) (2002), Quality is described as "the ability of a set of inherent characteristics of a product, system or process to fulfill the requirement of customers and other interested parties". These inherent characteristics can also be called "attributes". For coffee, the definition of quality and the attributes considered have probably evolved through the centuries. But nowadays, this definition varies along the production-to-consumer chain (Leroy *et al*., 2006). i.e.; at the farmer level, coffee quality is a combination of production level, price, and easiness of culture; at the exporter or importer level, coffee quality is linked to bean size, lack of defects and regularity of provision, tonnage available, physical characteristics and price; at the roaster level, coffee quality depends on moisture content, stability of the characteristics, origin, price, biochemical compounds and organoleptic quality (Leroy *et al*., 2006).

According to the definition of quality and standards authority of Ethiopia (QSAE) (2000), a quality is a conformance with requirements or fitness for use in which the parties involved in the industry customer, processor, supplier, etc.) Should agree on the requirements and the requirements should be clear to all stakeholders involved in the process. On the other hand,

Coffee Quality control and auction Center was established with a key objective of maintaining coffee quality control, which in turn facilitates the coffee marketing system to be standard based, and for the betterment /proper functioning of the long coffee supply chain of Ethiopia (Endale, 2008).

2.3.1. Factors affecting coffee quality

Coffee quality is a complex characteristic which depends on a series of factors among of this factors are; the species variety (genetic factors), environmental conditions (ecological actors), agro-economical practices (cultivation factors), processing systems (post-harvest factors), storage conditions, industrial processing, preparation of the beverage and taste of the consumer (Moreno *et al*., 1995).

Coffee quality is of critical importance to the coffee industry. It is a product that has desirable characteristics such as clean raw and roasted appearance, attractive aroma and good cup taste (Behailu *et al*., 2008). However, in Ethiopia, the quality of coffee produced by farmers has been deteriorating from time to time. Moreover, factors that determine coffee quality are genotypes, climatic conditions, and soil characteristics of the area, agronomic practices, harvesting methods and timing, post-harvest processing techniques, grading, packing, storage conditions and transporting, all contribute either exaltation or deterioration of coffee (Behailu *et al.*, 2008).

Similarly, Damanu (2008) reported coffee quality as a combination of the botanical variety, topographical conditions and climatic conditions and the care taken during growing, harvesting, storage, exports preparation, and transport. According to the author botanical variety and topographical conditions are constant and therefore dominate the inherent characters of a coffee whereas other factors except climatic conditions can be influenced by the human being and are a key factor in the determination of the end quality of a green coffee. Furthermore, inadequate systems of harvesting, storage, and transportation are responsible for the wide spread failure to maintain the inherent quality of coffee produced in Ethiopia (Alemayehu *et al*., 2008).

Climatic and Soil Factors: The environment has also a strong influence on coffee quality (Decasy *et al*., 2003). Altitude, daily temperature fluctuations, amount and distribution of rainfall and the physical and chemical characteristics of the soil are very important factors. Climate, altitude, and shade play an important role in temperature, availability of light and

water during the ripening period (Decasy *et al*., 2003). Rainfall and sunshine distributions have a strong influence on flowering, bean expansion, and ripening (Harding *et al.,* 1987).

The slower maturation process should, therefore, play a central role in determining high cup quality, possibly by guaranteeing the full manifestation of all biochemical steps required for the development of the beverage quality (Silva *et al*., 2005). For instance, chlorogenic acids and fat content have been found to increase with elevation in Coffee Arabica (Bertrand *et al*., 2006). Besides the beneficial effect of longer duration of the bean-filling period, a larger leaf area-to-fruit ratio (better bean-filling capacity) may also be linked to superior cup quality (Vast *et al*., 2006). The role of soil types has been well studied and it is generally admitted that the most acidic coffee quality is grown on rich volcanic soils (Harding *et al*., 1987).

Pre-Harvest and Harvest Factors: According to Bekele (2005) who reported that in South America, coffee grown with a heavy application of nitrogen fertilizer had poorer, lighter and thinner quality than that from unfertilized fields. An excess of nitrogen increases the caffeine content, resulting in a more bitter taste of the brew. The caffeine and chlorogenic acid contents of the beans are not affected by the levels of phosphorus, calcium, potassium, and magnesium in the soil. A lack of zinc will lead to the production of small light grey-colored beans, which will produce poor liquor (Wintgens, 2004). On the other hand, magnesium deficiency had an adverse effect on coffee cup quality (Mitchell, 1988). High concentration of calcium (>0.11%) and potassium (>1.75%) in the beans is associated with a bitter and "hard" taste (Wintgens, 2004).

The main factor affecting natural coffee quality is harvesting method. It is widely agreed that traditional hand-picking and husbandry labor, as opposed to mechanical harvest, produce the best quality green coffee by decreasing the percentage of defects in coffee batches. According to Bertrand *et al*., (2006) observed that yellow or green cherries picked at the end of the picking season contain beans with higher maturity level than red cherries of *Coffea Robusta* picked at the start of the picking season. This can be seen in bean size, chemical contents, and cup quality. On the other hand, for coffee Arabica in Costa Rica, early picking of red cherries gives the best coffee.

Genetic Factor: As harvesting method, post-harvest procedures and the physiology of the plant itself affect coffee quality, its genetic origin (species and genotype) also greatly influences coffee quality (Leroy *et al*., 2006). Similarly, Agwanda (1999) compared four

traits (acidity, body, and flavor) and overall standard for their suitability as selection criteria for the genetic improvement of overall liquor quality.

According to Bekele (2005), the coffee quality depends on genetic make-up and genes control the production of chemical compounds that behave as aroma agents either directly or as aroma precursors expressed during the roasting process. Hence while selecting a cultivar to be planted; cup quality must be the first priority to be considered. Furthermore, Moreno *et al*., (1995) improved that the cup quality of different coffee genotypes with the assistance of professional coffee tasters. Both researchers observed closely similarity among liquors in ranking various cup quality characteristics of the cultivars, indicating that anyone panel could have relied on selection for cup quality.

2.3.2. The chemical composition of coffee beans

The nonvolatile fraction of green coffee is composed primarily of water, carbohydrates and fiber, proteins and free amino acids, lipids, minerals, organic acids, chlorogenic acids, trigonelline and caffeine (**Table 3**).

Table 3: Chemical composition of roasted Coffee Arabica and Coffee Robusta seeds

Components	Concentration (g100 g^{-1})	
Carbohydrates/Fiber	Coffee Arabica	Coffee Robusta
Sucrose	6.0–9.0	0.9–4.0
Reducing sugars	0.1	0.4
Polysaccharides	34–44	48–55
Lignin	3.0	3.0
Pectin	2.0	2.0
Nitrogenous Compounds		
Protein/peptides	10.0–11.0	11.0–15.0
Free amino acids	0.5	0.8–1.0
Caffeine	0.9–1.3	1.5–2.5
Trigonelline	0.6–2.0	0.6–0.7
Lipids		
Coffee oil	15–17.0	7.0–10.0
Diterpenes (free and esterified)	0.5–1.2	0.2–0.8
Minerals		
Acids and esters	3.0–4.2	4.4–4.5
Chlorogenic acid	4.1–7.9	6.1-11.3
Aliphatic acids	1.0	1.0

Of these compounds found in green coffee, chlorogenic acids, caffeine, trigonelline, soluble fiber, and diterpenes from the lipid fraction are most likely to be bioactive, and they may also be important contributors to the beverage flavor after roasting (K¨olling-Speer and Speer, 2005).

The above (**Table 3)** deals with the main components of coffee bean such as caffeine, carbohydrates, chlorogenic acids, lipids, other nitrogenous compounds, volatiles, and include the transformation processes. Content varies according to cultivar, agricultural practices, climate, soil composition, and methods of analysis.

2.3.3. Lipids in coffee

Coffee oil is one of considered as an important vehicle to concentrate aroma and flavor of roasted coffee (Martin *et al.,* 2001). The coffee bean roasting significantly reduces levels of diterpene, increasing its stability and sensorial profile (Araujo and Sandi, 2007). According to Martin *et al.* (2001*)* the coffee oil composition, specifically, the fatty acids content can be considered as one of the chemical descriptors to differentiate between coffee varieties. In addition, Ramirez (2008) carried out in obtaining oil from roasted coffee with different extraction methods, reported the presence of saturated and unsaturated fatty acids, mainly palmitic, stearic, oleic, linoleic, and arachadic acids are the major fatty acids**.**

Furthermore, Folstar *et al*., (1985) demonstrated that the yield of lipids and fats can be obtainable in solvent extraction methods, depends on the particle size of the coffee is finely ground and types of solvents used. Speer (1989) extracted ground coffee of a particle size smaller than 0.63 mm and used tertiary butyl methyl ether as extraction solvent instead of the very dangerous diethyl ether. His method was adopted as a part of the DIN method 10779 (1999) and described as follows: roasted coffee beans are coarsely ground in a regular coffee mill and passed through a 0.63 mm sieve.5 g of the sieved material is then powdered together with sodium sulfate in a mortar and extracted with tertiary butyl methyl ether in a Soxhlet (4 h) siphoning 6-7 times per hour. The solvent is evaporated and the residue is then dried to constant weight (105°C). Longer extraction times (6, 8 or 10 hours) do not increase the lipid content.

2.3.4. Fatty acid and fatty acid in coffee

Fatty acids are aliphatic monocarboxylic acids with a large diversity in structure ranging from simple saturated carbon chains to more complex unsaturated, branched, and cyclic and cis/ trans configurations. They can also carry additional functional groups including keto, hydroxyl, peroxy and epoxy groups. Dicarboxylic acids do not occur in appreciable amounts in animal or vegetable lipids but can be produced metabolically from fatty acids and are useful industrial substrates. The fatty acids can be categorized into saturated monounsaturated and polyunsaturated fatty acids. The polyunsaturated fatty acids (PUFAs) can be further divided into omega-3 (ω-3), omega-6 (ω-6) and omega-9 (ω-9) types (Lercker *et al.,* 1996*).*

Unsaturated fatty acids which cannot be synthesized by humans and animals are known as essential fatty acids. This group includes five fatty acids, unsaturated oleic acid (C18:1), palmitic acid (C16:1), linoleic acid with two double bonds (C18:2), linolenic acid with three double bonds (C18:3) and, arachidonic acid which contains four double bonds (C 20:4) (Then *et al.,* 1996).

Fatty acids are present in the coffee lipid extract either free or mainly esterifies by glycerol or by diterpenic alcohols (Folstar *et al*., 1985). As the composition of fatty acids depends on several factors, particularly species and variety (Then *et al.,* 1996). The comparison of fatty acid patterns is a useful tool for classification purposes (Dagne *et al*., 1997). Main fatty acids present in the coffee oil are Myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), gondoic (C20:1) and behenic acid (C22:0) (Folstar *et al*., 1985; Lercker *et al.,* 1996). In particular, linoleic and α-linolenic acid was more abundant in Arabica coffee, while in Robusta contained a greater amount of oleic acid was observed (Rafael *et al*., 2014).

Folstar *et al*., (1975) and Speer *et al*., (1993) have investigated the fatty acids in the triacylglycerol of coffee beans and in the diterpene esters (Speer, 2001). Separation of the different lipid classes was achieved by Florisil column Speer *et al*., (1993) isolated the triacylglycerol by means of gel permeation chromatography; trans-esterified them with potassium methylate and chromatographic the methylated fatty acids using a 60 m fused silica capillary column.

Some reports indicated that during roasting there were only small changes in the fatty acid composition (Vitzthum, 1976). As Alves *et al.,* (2003) reported that in Arabica and Robusta

coffee the roasting process increased the trans-fatty acid levels, specifically the contents of C18:2ct and C18:2tc. According to Folstar (1975) and Speer (1993) reported, nine different free fatty acids were detected, which are similarly distributed in the Robusta and Arabica coffees, respectively.

In both coffee species (**Table 4),** the main fatty acids in both coffee species the main fatty acids were C18:2 and C16:0. It was also possible to detect large proportions of C18:0, C18:1, C20:0 and C22:0 but only minor traces of C14:0, C18:3 and C24:0. While the proportion of stearic acid is noticeably smaller than that of oleic acid in the Robusta, the percentages of these two acids in the Arabica coffees are almost equal. Feldman *et al*., (1969) stated that the lipid content in coffee grounds ranges from 10 to 17% of the total dry mass. However, compared to Coffee canephora, higher lipid contents are found in Arabica coffees. The majority of lipids are found in the oil fraction of the coffee bean endosperm. The coffee oil fraction is mainly composed of triacylglycerols, which have fatty acid proportions similar to those found in edible vegetable oils (Kölling-Speer and Speer, 2006).

Table 4: Fatty acids in triacylglycerol of green coffee beans (%)

Name of fatty acid	Folstar(1975)from waxed green beans	Speer (1993)	Speer (1993)
		Robusta (n=9)	Arabica (n= 4)
C14:0	0.2	Trace	Trace
C15:0	Trace	Trace	Trace
C16:0	33.3	27.2-32.1	26.6-27.8
C16:1	Trace	Trace	Trace
C17:0	Trace	Trace	Trace
C18:0	7.3	5.8-7.2	5.6-6.3
C18:1	6.6	9.7-14.2	6.7-8.2
C18:2	47.7	43.9-49.3	52.2-54.3
C18:3	1.7	0.9-1.4	2.2-2.6
C19:0	Trace	Trace	Trace
C20:0	2.5	2.7-4.3	2.6-2.8
C20:1	Trace	0.2-0.3	Traces-0.3
C21:0	Trace	Trace	Trace
C22:0	0.5	0.3-0.8	0.5-0.6
C23:0	Trace	Traces	Traces
C24:0	Traces	0.3-0.4	0.2-0.4

Accoriding to Bertrand *et al.,* (2008) investigated the possibility of using fatty acid composition as determined by gas chromatography (GC), Linoleic acid was the most

abundant fatty acid in the beans and accounted for 48−57% of the total fatty acid contents. In a review by Speer and Kolling-Speer (2006), the level of linoleic acid in Arabica green coffee beans was indicated at 52−54% of the total fatty acid. Similarly, Speer *et al., (*1993) reported arranging of 52.2-54.3% of this acid in Brazilian coffee. According to Villarreal *et al*. (2009) reported that the value of linoleic acid 41-46% of the total oil accounted for linoleic acid in Colombia coffee.

Esterification, the conversion of fatty acids into methyl esters, is commonly used to analyze fatty acids and to reduce the adsorption of solutes on the support and the surface of the column and improve compound separation (Gutnikov, 1995).

As Speer and Kolling-Speer (2006) mentioned that, the levels of palmitic acid range from 26−28% of the total fatty acids for green Arabica beans. However, Villarreal *et al*., (2009) reported a range of 31−35% and similarly Bertrand *et al., (*2008) reported arranging of 32 - 35% for palmitic acid in green Arabica beans from Colombia. However, Speer K *et al., (*1993) reported the range of 26.6-27.8% green Arabica beans from Brazil.

Table 5: Fatty acid compassion (%) in the green coffee bean in different countries.

	Coffee Farming Countries		
Fatty Acid Type	Colombia	Brazil	Reunion Island
Palmitic Acid	31−35	26.6-27.8	35
Myristic Acid	Trace	Trace	-
Linoleic Acid	41−46	52.2-54.3	44
Oleic Acid	8−12	5.6-6.3	7
Stearic Acid	6−8	6.7-8.2	7
Gondoic Acid	Trace	Trace	0.3
Behenic Acid	0.5-0.8	0.5-0.6	0.5
Source	Villarreal *et al.,* (2009)	Speer (1993)	Joet *et al.,*(2010)

Comparison of the concentrations (percentage by weight of total fatty acids) of individual fatty acids determined in green coffee beans of the Arabica variety from in three countries. From the above **Table 5** observed variations in the fatty acid contents between the green coffee beans from the different countries can be ascribed to several factors, including genetic traits, harvesting and postharvest processing methods, agricultural practices and environmental growing conditions (Villarreal *et al*., 2009).

3. METHODOLOGY

3.1. Description of the Study Area

The study was conducted in Amhara region, Ethiopia. specifically, West Gojjam, East Gojjam and Awi zones (**Figure 2**).

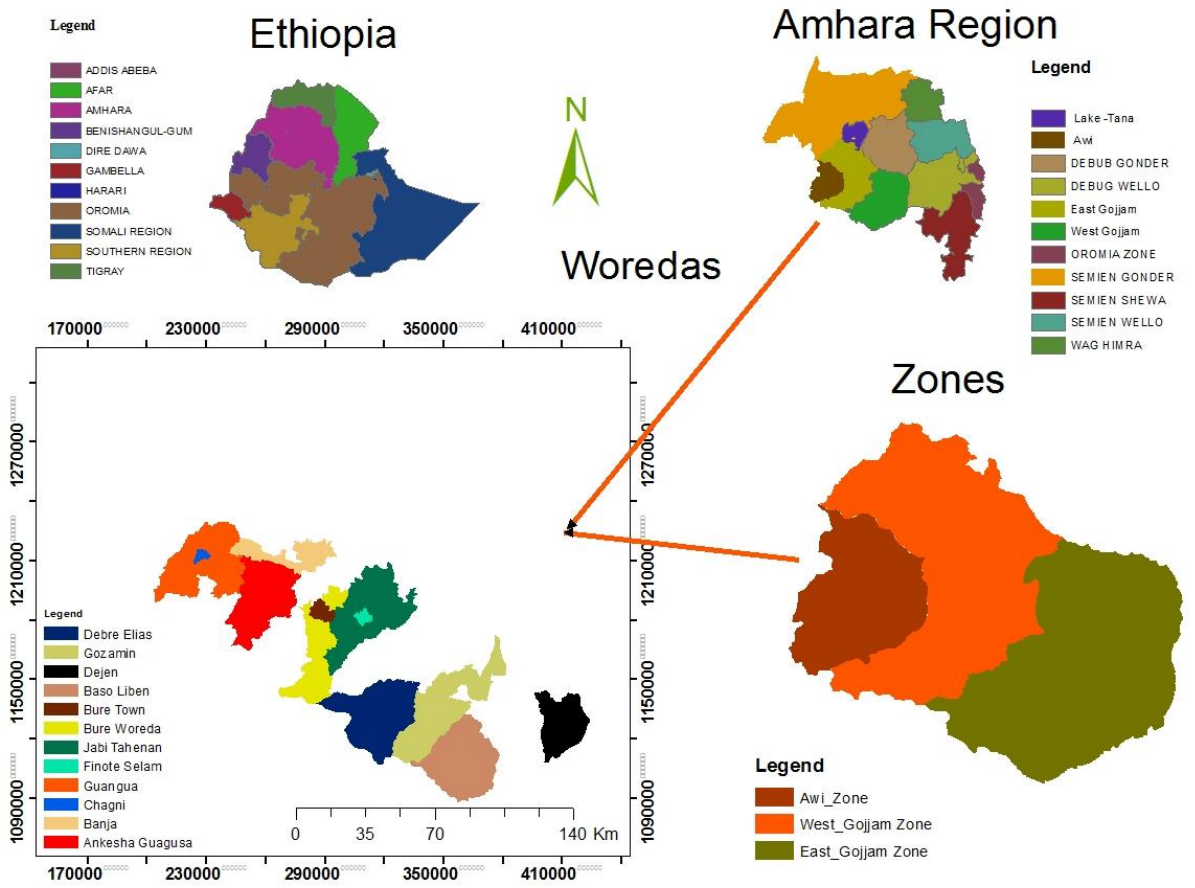


Figure 2: Location map of Ethiopia, Amhara region, West Gojjam, East Gojjam, and Awi zones with woredas (districts) depicting the coffee sample collection.

3.1.1. West Gojjam Zone

West Gojjam is one of the zone in Amhara region which lies between 36°30’ to 37°5’ Longitudes East and $10^0$16’ to $11^0$54’ Latitudes North. This is bordered on the south by the Abay River which separates it from the Oromia Region and Benishangul-Gumuz region, on the west by Agew Awi, on the northwest by North Gondar, on the north by Lake Tana, and the Abay River which separates it from the South Gondar, and on the east by East Gojjam. The capital city, Finoteselam is located between Addis Ababa and Bahir Dar which is 385 Km from Addis Ababa and 175 Km from the regional capital, Bahir Dar. Bahir Dar, Bure, Foteselam and Adet are some of the major towns in the zone. This zone includes Bahir Dar,

Adet, and Finote Selam, Bure, Quarite, Dega Damot, Sekela, Merawi, Yismala and Dembecha woredas (https://en.wikipedia.org/wiki/West_Gojjam_Zone).

Agriculture is the mainstay of farmers in the district which is characterized by mixed crop-livestock production systems. According to WGZARDO (2014), the most important crops grown in the district are cereals like wheat, teff, maize, barley, and oats. Pulse crops such as horse beans and chickpeas are produced. Oilseed crops (linseed and Niger seed), Vegetables (onion, garlic, coffee, potato, tomato, pepper, and carrot) and fruits (banana, mango, papaya, orange, and lemon) are also produced in the district. Green coffee beans are farming in different West Gojjam woredas (**Figure 2**). Specifically, Bure Zuria woreda, Bure city administration, Finoteselam city administration and JabieTehnan woreda are four major potentials of this zone to growth green Coffee Arabica. However, these coffees are of low monetary value, since they are still only consumed by people from the area (WGZARD, 2014).

3.1.2. East Gojjam Zone

East Gojjam is one of the zones of Amhara regional state, which is located in the northwest 300 km distance from Addis Ababa. It is bordered by South Gondar in the north, South Wollo in the east, North Shoa in the south east, East Wollega in the southwest and West Gojjam in the west. The Abbay River is the border of East Gojjam from the northeast through to the east, up to southwest and Debre Markos is the capital of the East Gojjam zone. (https://en.wikipedia.org/wiki/East_Gojjam_Zone).

The production of agriculture in this zone's district which is characterized by mixed crop-livestock production systems. According to the report by EGZARDO (2014), the most important crops grown in the district are cereals like wheat, teff, maize, barley, oats, Pulse and crops such as horse beans and chickpeas are produced. Oilseed crops (linseed and Niger seed), Vegetables (onion, garlic, potato, tomato, coffee, pepper, and carrot) and fruits (banana, mango, papaya, orange, and lemon) are also produced in the district. Therefore, Gozamen, Debre Elias, Degen and Basoliben woredas are four major potentials of this zone to growth green coffee Arabica is shown below. Green coffee beans are farming in different East Gojjam Woredas (**Figure 2**). Particularly, Basoliben woreda, Degen woreda, Debre Elias woreda and Gozamen woreda, are the major potentials of this zone to growth green Coffee Arabica. However, these coffees are of low monetary value, since they are still only consumed by people from the area (EGZARDO, 2014).

3.1.3. Awi zone

Agew Awi is one of the zones in the Amhara region of Ethiopia. It is located at the coordinates 10°51'N 36°47'E and altitude between 1,800 m - 3,100 m (on the nearby hills and mountains) with a total area of 131,844 ha. Agew Awi is bordered on the west by Benishangul-Gumuz Region, on the north by Semien Gondar zone and on the east by West Gojjam. The administrative center of Agew Awi is Injibara; other towns include Chagni and Dangila (Cherenet *et al.*, 2004).

Crop production, livestock farming, and forestry are the main sources of livelihood of farmers in different woredas in this zone. Different types of crops are produced namely, potato, teff, maize, wheat, barley, finger millet and other crops like bean, oats, onion, cabbage, coffee and other vegetables. The major crops produced in the area in the order of area coverage include potato, teff, and maize which cover 9200, 5800, 4000 and 6350 hectares, respectively. Some farm households use irrigation, particularly for coffee production which is the main vegetables produced (**Figure 2**). Specifically, Banja, Ayehu Guagusa, Guanghua and Chagni are the main farming woredas. However, these coffees are of low monetary value, since they are only consumed by people from the area. Raising cattle, sheep, and horse is a key economic strategy. Drought power is provided mainly by horse, which is cheaper to maintain than oxen. Sheep and cattle are the most commonly sold livestock type (AZARDO, 2015).

3.2. Sampling Techniques and Sample Size

Three zones were selected from Amhara region assigned to ensure representation of the producing areas, namely East Gojjam, West Gojjam and Awi zones (**Figure 2**). Within each of the three sampling zones, smaller sampling areas were assigned such as Dejen, Basoliben, Debre Elias, and Gozamen Woredas in under East Gojjam; JabieTehnan woreda, Finoteselam city administration, Bure Zuria woreda and Bure city administration in under West Gojjam and Guangua, Chagni, Ayehu Guagusa and Banja woredas in under Awi zone were selected, and from each, 250 g samples of green beans were received field collected directly from farms. Accordingly, a total of twenty four samples, twelve from East Gojjam zone, six from West Gojjam zone and five from Awi zone, were collected during the 2018 crop season by using judgmental sampling technique. All coffee samples are of low monetary value since they

are only consumed by people from the area. All of the green bean samples were obtained from ripe coffee cherries.

The coffee cherries were dried under at room temperature and separated beans and cherries were using mortal and pistol **(Figure 3).** All of the green coffee beans were subjected to room temperature dried just before grinding to fined powder by using a hand-held electrical Blade coffee grinder. The dried green coffee beans were ground to fine powered by using a hand-held electrical Blender coffee grinder, sieved with 200-micron mesh, and then powder was immediately packed in a plastic cup with a tight stopper and kept at room temperature until laboratory analysis.

Figure 3: Separation coffee beans from husks were using mortal and pistol

3.3. Chemicals and Apparatus

3.3.1. Apparatus

Flat form Shaker (Bench top Shaker, ZHWY-304/334/344), centrifuge (portal centrifuge, Japan), Plastics Bag, GC-MS (Agilent Technologies 7890B-5977A, China), Beaker, Electrical Girder (FW-100, High Speed Universal Disintegrator girders), Oven (DAIHAN scientific natural flow type oven), Balance (RADWAG:ps360/c/1), Round Bottle Flask, Spoon, Vials, Incubator (constant temperature and humidity incubator), Vacuum Rotary evaporator (Stone Staffordshire,England,ST15 0SA), Mortar, Pistol, Test Tube, Micro Pipit (china), Refrigerator (digital inverter technology, Samsung), syringe, membrane filter, and Crimper (crimper tool 11mm hand crimper,QTY:1) were used for laboratory analysis.

3.3.2. Chemicals

All reagents and standards that were used in the analysis were of analytical grade. Standard of fatty acids was used for the laboratory analysis. Other chemicals; Methanol (absolute acetone free ,India), chloroform (99.99%, 40005-India), toluene (99.99%,Thailand), Chromatographic

grade *n*-hexane (99.9%, France), acetone (99.5% ,20020-ARESE(Ml)), sulfuric acid (98%,40005-India), anhydrous sodium sulfate (99%,133001-India), sodium chloride (99.5%,121001-Blulux laboratory) and Nervonic acid (99%, 16823-0048USA) as internal standard was used for the laboratory analysis.

3.4. Sample Preparation for GC-MS Analysis

3.4.1. Extraction of lipids

Lipids from green coffee beans were extracted according to the method of Folch *et al.,* (1957) with some modifications. Briefly, 0.500 g portion of coffee powder was extracted with 8 ml chloroform and 4 ml methanol (2:1 ratio v v^{-1}) by shaking for 36 h on a platform shaker with aid of test tube at 300 rpm. The extract was centrifuged and the filtrate was taken. The lipid phase was separated with the aid of 2 ml of 0.73% aqueous sodium chloride, then the upper phase was removed by using micropipette (siphoning) and the lower phase (chloroform) layer containing the lipid was recovered (**Figure 4**). The solvent was removed under vacuum rotary evaporator and the residue was washed in 5.0 mL of toluene.

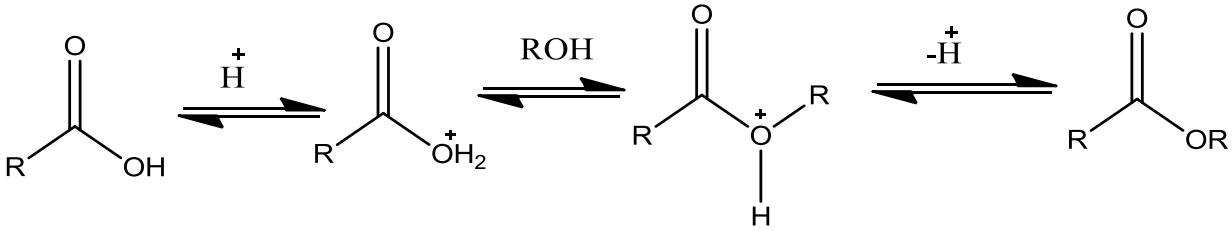
a  **b** 

Figure 4: Extraction of lipids on platform shirker (a) and extracted lipids(b) from green coffee beans

3.4.2. Derivatization of fatty acids

The analysis of fatty acids by GC is complicated by their polarity and inadequate volatility. To permit analysis by GC, the polar carbonyl groups must first be converted to produce more volatile non-polar derivatives. A wide range of alkylation reagents are available for this purpose and fatty acids are frequently converted to their corresponding fatty acid methyl esters (FAMEs). FAME derivatization has been extensively used for fatty acid analysis, especially in connection with mass spectroscopy detection (William and Dewar, 1958).

In **Scheme** below**,** Fatty acids were converted to the corresponding methyl esters with aid of sulfuric acid, methanol, and heat prior to GC-MS analysis Christie WW (1993).



Scheme 1: **General mechanism of acid-catalyzed esterification of fatty acids.**

Briefly, a 1.0 mL portion of the lipid extract in toluene was spiked with 40 µL of 0.57 mg ml^{-1} nervonic acid and allowed to react with 2.0 mL of 1% methanolic sulfuric acid solution for 12 h, while maintained at 50^{o} C in an incubator. After that reaction, the mixture was treated with 5 ml of 5% aqueous sodium chloride solution and the required ester was extracted with hexane (2×3 ml) after phase separation, then the upper phase was taken away by using micropipette (siphoning) and dried over anhydrous sodium sulfate then transferred in to the vial and analyzed by GC-MS.

3.5. GC-MS analysis

An Agilent Technologies 8790A gas chromatographic system equipped with an autosampler, a split splitless injector, and a mass spectrometer (Agilent Technologies 7890B-5977A) was used for GC-MS analysis of the fatty acid methyl esters. The chromatographic conditions were: 1µL sample injection volume (split less), injector temperature 280^{o} C, a DB-5 MS fused silica capillary column (30m x 250 µm x 0.25 µm; Agilent Technologies, China), temperature program conditions of 40°C (held for 3 min), then ramped at 5°C mini $^{-1}$ to 230° C (held for 20 min), helium was used as carrier gas at a flow rate of 1.68 mL min^{-1}. Conditions used for the MS were: transfer temperature 300^{o} C, scan range mz^{-1} 60−400, ionization potential 70 eV and electron multiplier voltage 3000 V.

3.6. Quantification of Fatty Acids

The concentrations of twenty three fatty acids were detected whose percentages higher than 0.01% relative peak area of the total fatty acid. However, only thirteen fatty acids, with relative percentages of peak area higher than 0.1%, were quantified accurately and used for geographical origin comparison of the coffee sample types. These fatty acids: Myristic, pentadecanoic acid, palmitic, margaric, stearic, linoleic, oleic, 14-methyleicosanoic acid,

gondoic, tricosanoic acid, arachidic, behenic and lignoceric acids, were determined relative to the internal standard by using the following equation (Dussert, 2008).

$$\boldsymbol{W / W(mg/g) = (AFA \times MIS)/(AIS \times MC)}$$

Where **AFA** is the peak area of the fatty acid, **AIS** is the peak area of the internal standard, **MIS** is the mass of the internal standard and **MC** is the mass of coffee sample used for the analysis.

3.7. Statistical Analysis

Data analysis was performed by using the statistical software packages SPSS 22 (IBM Corp, USA). Each dataset consisted of a matrix, in which the columns represented the individual green coffee bean samples, and the rows consist of the chromatographic peak areas of all the detected fatty acids, or the concentrations of fatty acids determined. One-way analysis of variance (ANOVA) was used to test for the presence of significant differences in the mean concentrations of fatty acids among the Zones and Woredas types of green coffee beans. Differences were considered significant when $p < 0.05$. Whenever necessary, box plots (box and whisker plots) were constructed to display the differentiation among coffee types. Samples were extracted and analyzed in triplicate, and average values were used for statistical calculations.

4. RESULTS AND DISCUSSION

4.1. Characterization of Fatty Acids

A total of twenty three fatty acids, sixteen saturated and seven unsaturated fatty acids were detected in all of the green coffee bean samples (**Figure 5**). The identities of the detected fatty acids were determined by using the NIST-MS spectral library as a reference (**Table 6**).

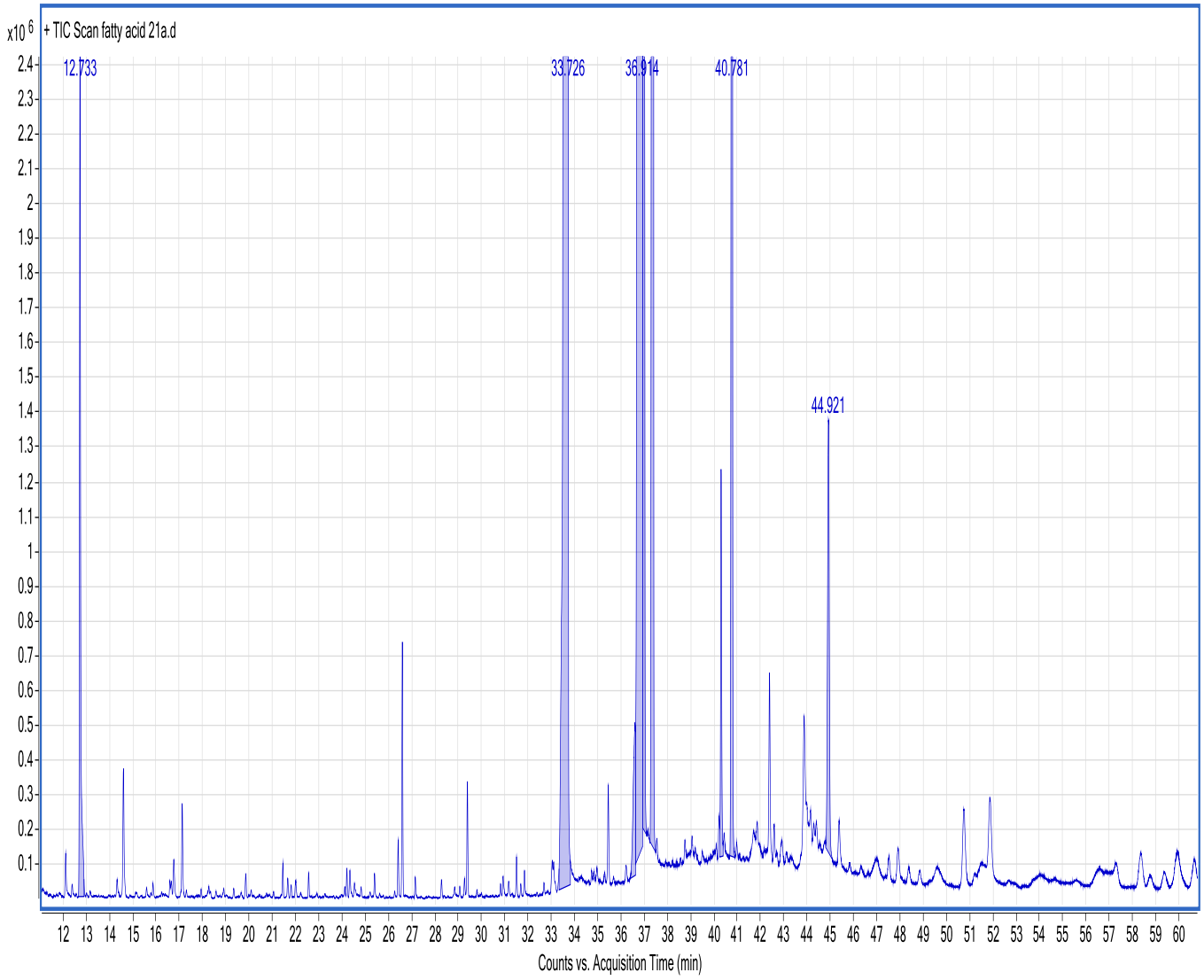
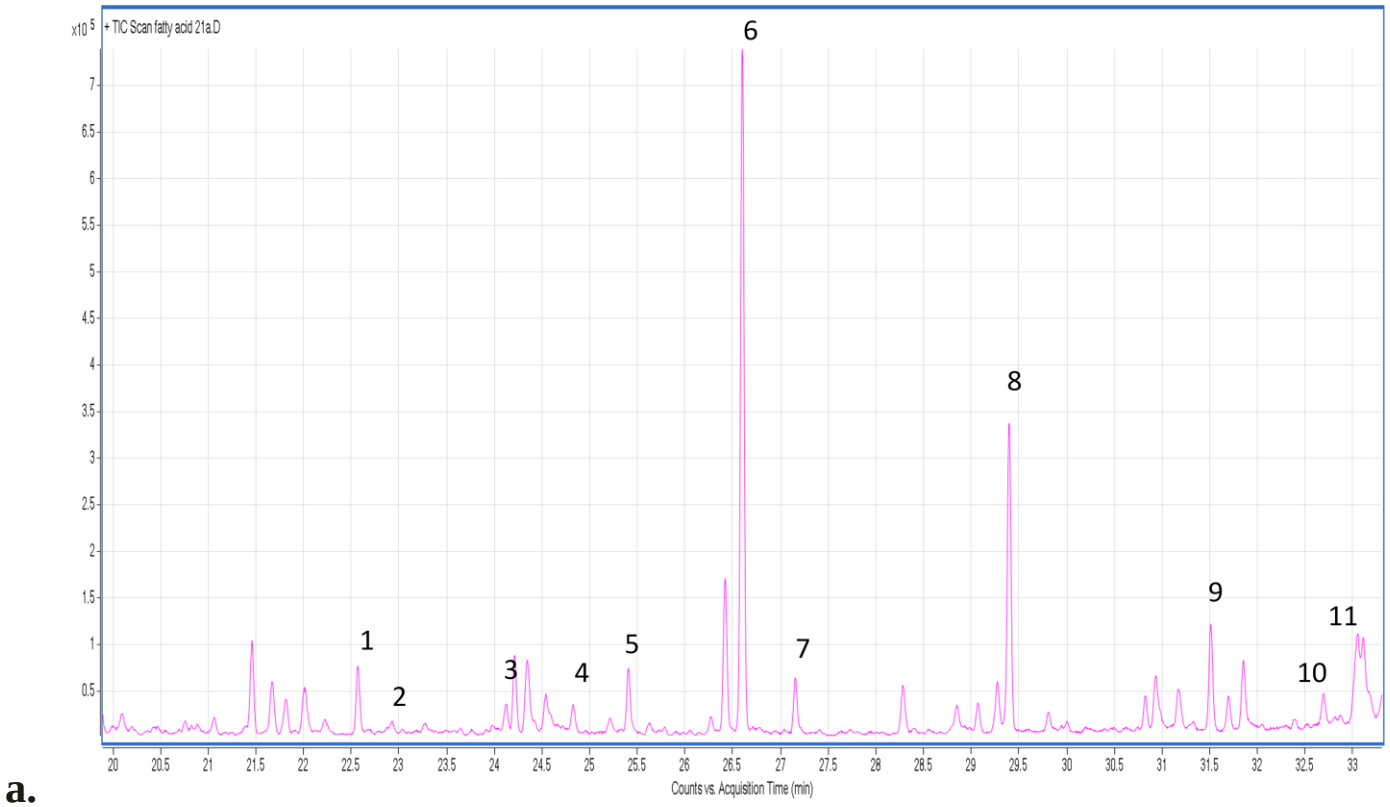
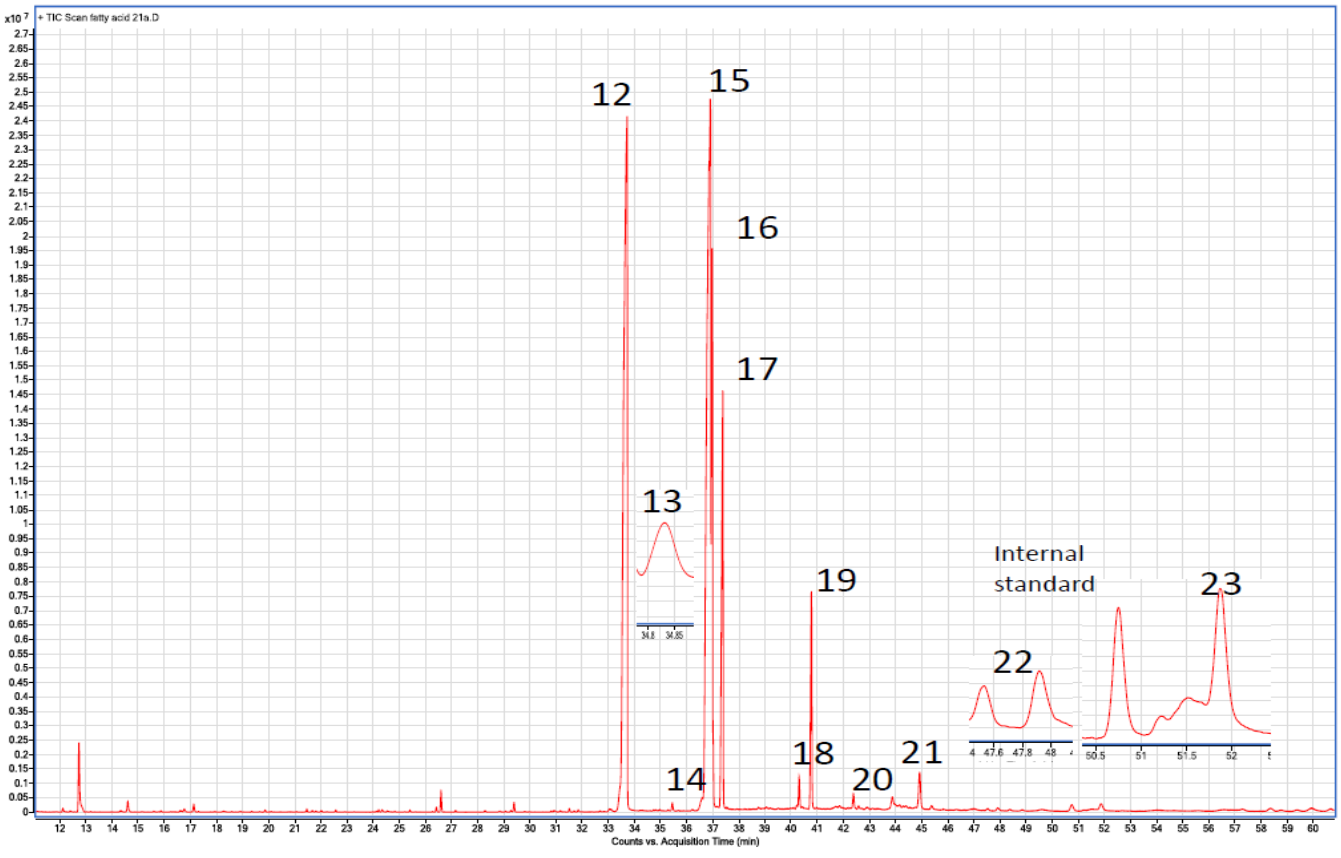


Figure 5: A typical GC-MS chromatogram of East Gojjam zone green coffee bean extract, indicating the twenty three detected fatty acids.



a.



b.

Figure 6: GC-MS chromatogram of twenty three fatty acid 1-11 (**a**) and 12-23 with an internal standard of nervonic acid (**b**) in green coffee beans sample lies out from (**Figure 5)** chromatogram.

Table 6: The names, molecular formula, retention times (RT) and means of identification of the fatty acids determined in the green coffee beans.

No	IUPAC Name	Common Name	Molecular Formula	Means of Identification	Retention Time
1	9-Oxononanoic Acid	Azelaaldehydic Acid	$C_9H_{16}O_3$	NIST-MS	22.567
2	Octanedieoic Acid	-	$C_8H_{14}O_4$	NIST-MS	22.928
3	6,6-DimethoxyOctanoic Acid	8,8-Dimethoxyoctanoic	$C_{10}H_{20}O_4$	NIST- MS	24.213
4	10-Methylundecanoic	-	$C_{12}H_{24}O_2$	NIST- MS	24.824
5	Nonanedioic Acid	Azelaic Acid	$C_9H_{16}O_4$	NIST- MS	25.402
6	12,12-Dimethoxdodecanoic Acid	-	$C_{14}H_{28}O_4$	NIST- MS	26.6
7	3,7,11-Trimethyl-8,10-Dodecedienic Acid (Z,Z)	-	$C_{16}H_{28}O_2$	NIST- MS	27.153
8	Tetradecanoic Acid	Myristic Acid	$C_{14}H_{28}O_2$	NIST- MS	29.4
9	Pentadecanoic Acid	Pentadecylic Acid	$C_{15}H_{30}O_2$	NIST- MS	31.5
10	9,12-Octadecadienoic Acid (E,E)	-	$C_{18}H_{32}O_2$	NIST- MS	32. 7
11	9-Hexadecenoic Acid (Z)	Palmitoleic	$C_{16}H_{30}O_2$	NIST- MS	33.1
12	Hexadecanoic Acid	Palmitic Acid	$C_{16}H_{32}O_2$	NIST- MS	33.726
13	17-Octadecynoic Acid (Z)	-	$C_{18}H_{32}O_2$	NIST- MS	34.834
14	Heptadecanoic Acid	Margaric Acid	$C_{17}H_{34}O_2$	NIST- MS	35.452
15	9,12-Octadecadienoic Acid (Z,Z)	Linoleic Acid	$C_{18}H_{32}O_2$	NIST- MS	36.914
16	9-Octadecenoic Acid (Z)	Oleic Acid	$C1_8H_{34}O_2$	NIST- MS	36.982
17	Octadecanoic Acid	Stearic Acid	$C_{18}H_{36}O_2$	NIST- MS	37.388
18	11-Eicosenoic Acid (Z)	Gondoic Acid	$C_{20}H_{38}O_2$	NIST- MS	40.3
19	Eicosanoic Acid	Arachidic Acid	$C_{20}H_{40}O_2$	NIST- MS	40.78
20	14-Methyleicosanoic	-	$C_{21}H_{42}O_2$	NIST- MS	42.582
21	Docosanoic Acid	Behenic Acid	$C_{22}H_{44}O_2$	NIST- MS	44.917
22	Tricosanoic Acid	-	$C_{23}H_{46}O_2$	NIST- MS	47.92
23	Tetracosanoic Acid	Lignoceric Acid	$C_{24}H_{48}O_2$	NIST- MS	51. 859

NIST- MS = National Institute of Standards and Technology-Mass Spectroscopy

4.2. Fatty Acid profile of Coffee Beans at Zonal level

The fatty acid compositions of the coffee samples from the three zones studied are given in (**Table 7)**. The total fatty acid contents of the green coffee beans ranged from 67.89-136.71 mg g^{-1} in samples in the three zones.

Linoleic acid was the most abundant fatty acid in the beans which accounted for 29.37-46.64 mg g^{-1} of the total fatty acid contents in this study. Speer and Kolling-Speer (2006) who reported that the levels of linoleic acid ranged from 52.2−54.3 mg g^{-1} of the total fatty acids for green coffee Arabica beans collected from different geographical origins of Brazil. The results of this study were found below the finding of the previous report.

The level of palmitic acid was the second most abundant fatty acid, representing 26.32-40.43 mg g^{-1} of the total fatty acids in the present study. The result was in agreement with Villarreal *et al.,* (2009) who reported that the palmitic acid of green coffee Arabica beans from Colombia ranged from 33−36 mg g^{-1} However, According to Speer and Kolling-Speer (2006), the levels of palmitic acid ranged from 26.6−27.8 mg g^{-1} of the total fatty acids for green coffee Arabica beans collected from different geographical origins of Brazil; which is lower than the present study. The two fatty acids (Linoleic and Palmitic acid) comprised 69.14-81.01 mg g^{-1} of the total fatty acid content measured in the green coffee beans. Whereas oleic acid, stearic acid and arachidic acid were constituent 4.53-17.62, 5.34-14.58, and 1.44-5.0 mg g^{-1} respectively, of the total fatty acids.

Based on the analysis of variance there was significant difference ($p<0.05$) between zones. The result revealed that the higher (102.74 mg g^{-1}) fatty acid concentration was found in West Gojjam zone while the lower (84.56 and 90.55 mg g^{-1}) fatty acid concentration was found in East Gojjam zone and Awi zone. However, the Tukey analysis showed that there is no significant difference ($p>0.05$) between East Gojjam and Awi zones **(Table 7).**

From the major fatty acids, linoleic acid was significantly higher in samples from West Gojjam zone (46. 64 mg g^{-1}) than in those from East Gojjam (42.75 mg g^{-1}) and Awi zones (39.08 mg g^{-1}). On the other hand, from a lower concentration of total fatty acid, Myristic acid (0.12-0.2 mg g^{-1}) was significantly higher in West Gojjam green coffees beans than coffee from other zones (0.10-0.15 mg g^{-1}). However, pentadecanoic acid, margaric acid, oleic acid, gondoic acid, behenic acid, 14-methyl eicosanoic acid and lignoceric acid presents in all three zones green coffee beans have no significant difference.

Table 7: The mean, standard deviation, minimum (Min) and maximum (Max) concentrations (mg g^{-1}) of fatty acids were found in green coffee beans from West Gojjam, Awi zone, and East Gojjam zone.

ZONES AND NUMBER OF SAMPLES										
Fatty Acids	West Gojjam(n=7)			Awie zone(n=5)			East Gojjam(n=12)			
	Mean	Min	Max	Mean	min	max	Mean	min	Max	p
C14:0	0.15±0.023[a]	0.12	0.2	0.13±0.01[b]	0.12	0.15	0.12±0.01[b]	0.10	0.15	*
C15:0	0.13±0.008	0.10	0.16	0.12±0.003	0.08	0.16	0.11±.006	0.11	0.12	ns
C16:0	36.14±2.36[a]	32.11	40.43	32.43±0.62[b]	29.83	35.13	30.90±3.49[b]	26.32	39.92	*
C17:0	0.49±0.088	0.13	3.06	0.17±0.012	0.15	0.19	0.15±0.02	0.11	0.19	ns
C18:2	40.3±3.79[a]	35.75	46.64	35.72±2.27[b]	33.32	39.08	33.20±3.76[b]	29.37	42.75	*
C18:1	9.752±2.59	7.22	17.62	9.28±0.98	8.01	10.71	8.51±1.45	4.53	11.84	ns
C18:0	9.74±2.15[a]	7.16	14.58	7.81±1.15[b]	6.73	9.85	7.32±0.98[b]	5.34	8.77	*
C20:1	0.41±0.11	0.13	0.58	0.41±0.03	0.36	0.46	0.38±0.099	0.04	0.52	ns
C20:0	3.92±0.68[a]	3.01	5.00	3.25±0.60[b]	2.68	4.46	2.86±0.47[b]	1.44	3.74	*
C21:0	0.32±0.096	0.04	3.67	0.05±0.009	0.04	0.06	0.046±0.007	0.03	0.06	ns
C22:0	0.85±0.212	0.03	1.52	0.91±0.18	0.68	1.27	0.75±0.13	0.46	0.96	ns
C23:0	0.10±0.025[a]	0.07	0.14	0.09±0.01[ab]	0.08	0.10	0.08±0.01[b]	0.05	0.10	*
C24:0	0.44±0.079	0.15	3.21	0.25±0.046	0.17	0.32	0.22±0.047	0.07	0.35	ns
Total	102.74±15	85.96	136.71	90.55±5.93	82.21	101.83	84.56±10.50	67.89	109.41	

P=probability level, significance at p<0.05 and ns = not significant at p>0.05, Means followed by the same letters in a row are not a significant difference (p<0.05).*

The concentration of the total fatty acid (mg g^{-1}) present in green coffee beans in the study of three zones were shown in the below (**Figure 7**). Those total thirteen fatty acids; myristric (C14:0), pentadecanoic (C15:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1n-9), linoleic (C18:2n-6), arachidic (C20:0), gondoic (C20:1n-9), 4-methyleicosanoic acid (C21:0), behenic acid (C22:0), tricosanoic (C23:0) and lignoceric (C24:0) acids were detected and quantified by GC-MS analysis. Among these, Myristric acid, palmitic acid, linoleic acid, stearic acid, arachidic acid and tricosanoic acid were distinguished at each zone, i.e. the concentration of those total fatty acids in samples from West Gojjam zone are higher than samples from Awi zone followed by in samples from East Gojjam zone. In addition, pentadecanoic acid, margaric acid, gondoic acid, behenic acid, and lignoceric acids were found to be trace in concentration.

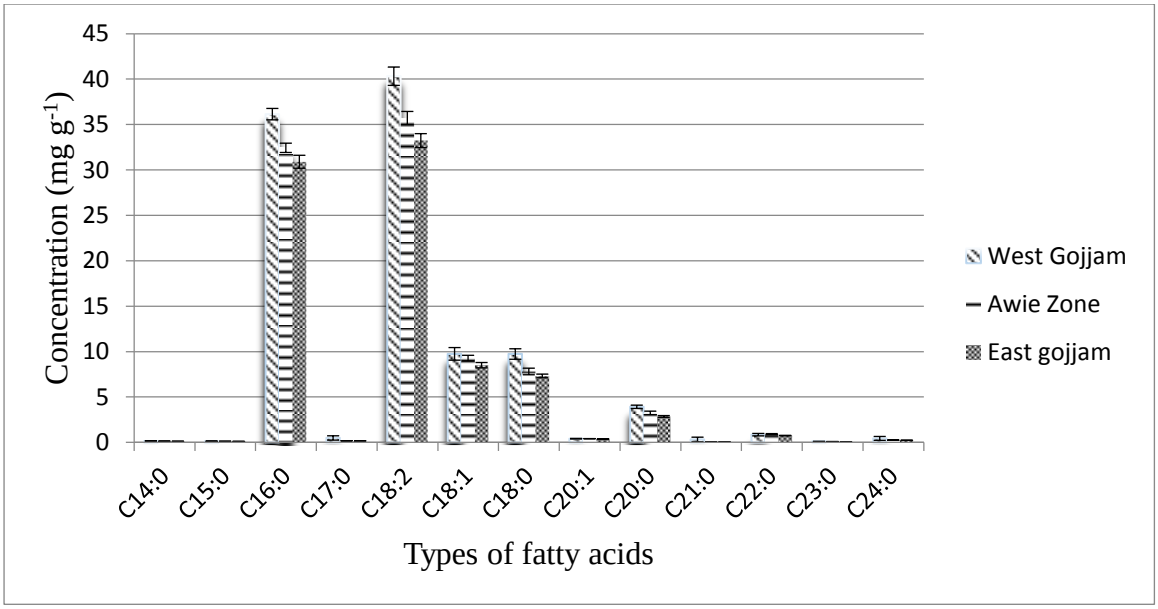


Figure 7: Fatty acid concentrations (mg g^{-1}) of green coffee bean in West Gojjam, East Gojjam and Awi zones samples.

The observed variations in the fatty acid contents between the green coffee beans from the three different zones can be ascribed to several factors, including genetic traits, harvesting and postharvest processing methods, agricultural practices and environmental growing conditions, which is similar with the finding of coffee beans from Colombia (Villarreal *et al*., 2009). However, differences in agricultural practices and harvesting methods are unlikely to explain the variations in the fatty acid contents (Amamo, 2014) because the traditional way of farming and harvesting, involving hand picking of ripe coffee cherries is used throughout of those zones. Regarding the postharvest processing conditions, the coffee samples were processed by dry methods in all the sampling area. However, the way the cherries are picked and drying the samples were different from farmers to farmers. Some farmers used to pick both the ripped and the un-ripped cherries while others have selected only the fully ripped (red cherries). Some farmers have followed the right procedures (well-designed beds) while others were used on the ground together with other spices. Several investigators have pointed out that genetic properties play of real role in determining the fatty acid content of green Arabica coffee beans (Bertrand *et al*., 2008; Villarreal *et al*., 2009). Although the existence of high genetic variability among coffees grown in different regions of the country has been established by several researchers (Lashermes *et al*., 1996; Tessema 2011), it was established that the fatty acid composition of green coffee beans is mainly controlled by the mean air temperature during seed development (Joet *et al*., 2010). This is particularly pertinent during the final five months before harvest (Joet *et al*., 2010). In similarly with this study, Villarreal

et al (2009) who reported that significant variation in the fatty acid compositions of green Arabica coffee beans from different regions and varieties in Colombia. Therefore; it can be anticipated that the fatty acid content of coffee beans grown in different zones of Amhara regions may vary due to genetic factors, postharvest processing methods and environmental conditions under which they are grown.

Green coffee beans from West Gojjam are distinguished from those from two zones mainly by their linoleic acid content (**Figure 8).** Based on the analysis of variance there were significant difference ($p<0.05$), the concentrations of linoleic acid found in green coffee beans from West Gojjam zone (46.64 mg g^{-1}) was significantly higher than coffee beans from the rest zones, namely, Awi zone (39.08 mg g^{-1}) and East Gojjam (42.75 mg g^{-1}). Furthermore, the concentration of linoleic acid content from each individual coffee samples found in West Gojjam zone was ranged 35.75−46.64 mg g^{-1} which is higher than those found in beans from Awi zone (33.32-39.08 mg g^{-1}) followed by East Gojjam zone (29.37-42.75 mg g^{-1}).

The contents of linolic acid in West Gojjam zone coffee beans higher than the rest two zones may be attributed to genetic, environmental factors or may be harvesting method. According to Bertrand *et al*., (2008) who reported that the contents of linoleic acid was one of the most discriminate parameters for coffee varieties differentiation between coffee Robusta and coffee Arabica due to genetic variation from Colombia coffee beans.

The box plot constructed from the concentrations of linoleic acid clearly separated West Gojjam zones green coffee beans from the rest two zones and used to show overall patterns of response. It provides a useful way to visualize the range and other characteristics of responses for a three zones. The box plot of linoleic acid in Awi zone was comparatively short. This suggests that overall linoleic acid have a high level of agreement with each other. The box plot was comparatively tall at West Gojjam and East Gojjam. This suggested that linoleic acid quite different opinions from the median value. The whole, one box plot is much higher or lower than another in below (**Figure 8)**. This could suggest a difference concentration of linoleic acid between zones.

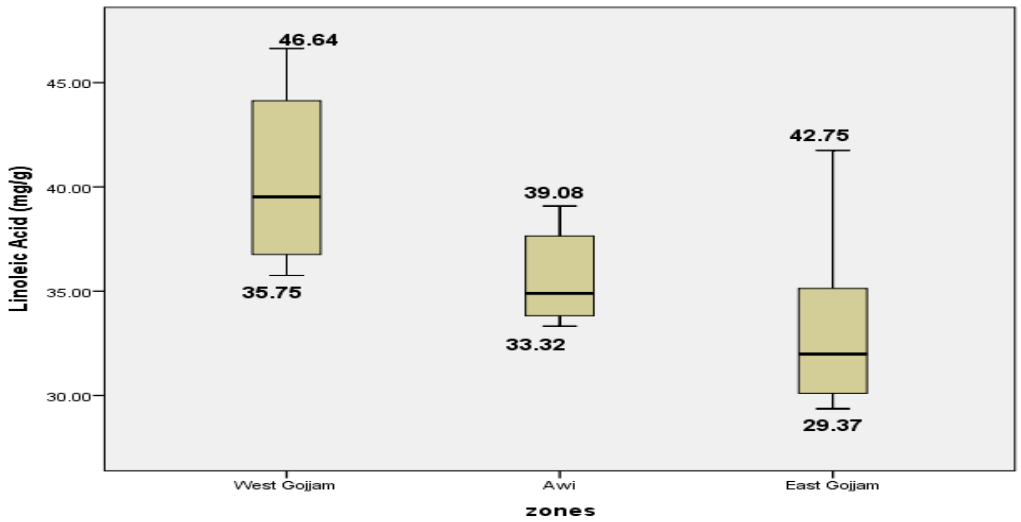


Figure 8: Box plot indicating the variation in the concentrations (mg g^{-1}) of linoleic acid between West Gojjam, East Gojjam and Awi zones green coffee beans.

4.3. Fatty Acid Profile of Coffee Beans in the Twelve Woredas

Statistical analysis using one-way ANOVA (p = 0.05) was performed to test the presence of significant differences in fatty acid concentration of coffee beans between the twelve Woredas (**Table 8**). Among those woredas, Bure city administration coffees from West Gojjam zone contained significantly higher (123.54 mg g^{-1}) total fatty acid content than the rest of woredas coffee samples (78.45-104.88 mg g^{-1}). These coffees notably contained higher concentrations of the major fatty acids, i.e. palmitic, linoleic, oleic and stearic acids and arachidic acid. Among this linoleic acid (46.64 mg g^{-1}) was found to be indicative for distinguishing all of these coffees from all other types. On the other hand, the concentration of linoleic acid significantly lower from the samples in Dejen, Basoliben, Debre Elias, Banja, Ayehu Guagusa, and Chagni, (31.41, 32,56, 33.18, 33.92, 32.92, and 34.37 mg g^{-1}) respectively than Bure city administration (46.64 mg g^{-1}). However, minor concentrations of fatty acids, such as; pentadecanoic acid, margaric acid, and gondoic acid were present in coffee beans, which are similar in all twelve Woredas.

Table 8: The mean, standard deviation, minimum (Min) and maximum (Max) concentrations (mg g^{-1}) of fatty acids found in green coffee beans from different woredas in West Gojjam, Awi and East Gojjam zones in Amhara regions.

zone	Coffee type		C14:0	C15:0	C16:0	C17:0	C18:2n-6	C18:1n-9
West Gojjam	Finote Selam	mean± std	0.12±0.001 ^{abc}	0.14±0.001	34.76±2.3 ^{abc}	0.14±0.005	38.06±2.1 ^{ab}	8.94±1.28 ^{b}
		min	0.12	0.13	32.11	0.13	35.75	7.76
		max	0.14	0.15	37.02	0.15	40.21	10.62
	Jabie Tehnan	mean±std	0.16±0.003 ^{ab}	0.15±.008	37.21±0.3 ^{ab}	0.89±1.45	42.53±2.8 ^{ab}	9.09±0.6 ^{b}
		min	0.13	0.14	36.84	0.14	39.52	8.4
		max	1.0	0.16	37.54	3.06	45.53	9.69
	Bure Zuia	mean±std	0.156±0.001 ^{abc}	0.15±.008	34.55±1.1 ^{abc}	0.17±0.03	37.4±1.4 ^{bc}	8.59±0.93 ^{b}
		min	0.15	0.14	33.6	0.14	36.22	7.22
		max	0.17	0.17	35.63	0.2	39.1	9.28
	B/city ad..	mean±std	0.16±0.002 ^{a}	0.155±0.003	39.97±0.64 ^{a}	1.06±1.2	46.64±0.6 ^{a}	15.0±3.7 ^{a}
		min	0.16	0.15	39.5	0.18	45.8	12.387
		max	0.164	0.16	40.4	1.9	46.6	17.6
Awie Zone	Guangua	mean±std	0.13±0.001 ^{abc}	0.13±00.01	32.77±0.1 ^{abc}	0.185±0.	39.18±0 001 ^{abc}	10.7±001 ^{b}
		min	0.13	0.14	32.76	0.18	39.08	10.7
		max	0.134	0.16	32.78	0.185	39.28	10.17
	Chagni	mean±std	0.13±0.002 ^{ab}	0.1 5±.001	32.02±0.9 ^{bc}	0.17±0.004	34.37±0.7 ^{c}	9.03±0.1 ^{b}
		min	0.1262	0.146	31.39	0.16	33.82	8.95
		max	0.1287	0.148	32.66	0.17	34.90	9.12
	Ayehu Guagusa	mean±std	0.12±0.005 ^{abc}	0.14±.002	31.27±1.3 ^{bc}	0.16±0.01	33.92±0.7 ^{c}	8.39±0.44 ^{b}
		min	0.118	0.14	29.83	0.15	33.32	8.10
		max	0.13	0.146	32.94	0.18	34.86	8.97
	Banja	mean±std	0.148±0.001 ^{abc}	0.14±.0002	34.82±0.43 ^{abc}	0.16±0.01	33.92±0.7 ^{c}	8.39±0.44 ^{b}
		min	0.14	0.13	34.52	0.15	33.32	8.0
		max	0.15	0.16	35.13	0.17	34.86	8.97
East Gojjam	Basolibe n	mean±std	0.12±0.001 ^{bc}	0.14±.004	30.56±2.17 ^{bc}	0.14±0.02	32.56±2 ^{c}	8.49±1.86 ^{b}
		min	0.11	0.137	27.43	0.13	29.85	4.53
		max	0.13	0.15	33.91	0.186	35.71	11.84
	Degen	mean±std	0.12±0.012 $^{b\,c}$	0.14±.008	29.07±2.4 ^{c}	0.14±0.028	31.41±2.1 ^{c}	7.49±0.76 ^{b}
		min	0.10	0.13	26.31	0.11	29.37	6.59
		max	0.12	0.15	31.53	0.17	33.43	8.27
	Deber Elias	mean±std	0.12±0.005 ^{bc}	0.14±.006	30.58±2.8 ^{bc}	0.14±0.008	33.18±3.72 ^{c}	8.68±0.91 ^{b}
		min	0.1	0.14	28.58	0.13	29.82	7.79
		max	0.15	0.15	34.24	0.15	38.10	9.83
	Gozamen	mean±std	0.13±0.001 ^{abc}	0.15±.006	34.06±6.4 ^{abc}	0.16±0.026	36.79±6.3 ^{bc}	9.29±1.28 ^{b}
		min	0.11	0.14	27.92	0.13	30.98	7.87
		max	0.14	0.16	39.92	0.19	42.75	10.45
		p	*	ns		ns	*	*

P=probability level, significance at p<0.05 and ns = not significant at p>0.05. Means followed by the same letters in a column are not a significant difference (p<0.05).*

zone	Coffee type		C18:0	C20:1n	C20:0	C22:0	C23:0	C24:0	Total
West Gojjam zone	Finote Selam	mean±std	7.75±0.63 ^{cd}	0.35±0.04	3.08±0.07 ^{def}	0.39±0.41 ^{c}	0.09±0.02 ^{abc}	0.18±0.03 ^{b}	93.90±6.9
		Min	7.16	0.31	3.01	0.13	0.07	0.15	86.64
		max	8.43	0.39	3.17	0.80	0.12	0.21	101.32
	Jabie Tehnan	mean±std	8.92±0.68^{bcd}	0.40±0.19	4.3±0.38 ^{ab}	0.93±0.64 ^{abc}	0.11±0.03 ^{ab}	0.29±0.09 ^{b}	104.88±7
		Min	8.49	0.13	3.9	0.05	0.07	0.18	97.85
		max	9.93	0.58	4.8	1.5	0.14	0.35	113.39
	Bure Zuia	mean±std	11.18±2.3 ^{ab}	0.44±0.09	3.89±0.32 ^{bcd}	0.96±0.1^{abc}	1.0±0.02 ^{abc}	0.23±0.07 ^{b}	97.72±6.5
		Min	9.35	0.36	3.48	0.08	0.08	0.18	90.89
		max	14.58	0.54	4.14	1.07	0.12	0.3	105.20
	B/city ad..	mean±std	12.43±0.4^{a}	0.47±0.001	4.90±0.14 ^{a}	1.43±0.13 ^{a}	0.12±0.023 ^{a}	1.71±2.12 ^{a}	123.54±9
		Min	12.12	0.466	4.8	1.3	0.11	0.2	117.07
		max	12.74	0.477	5.0	1.5	0.14	3.2	129.76
Awi Zone	Guangua	mean±std	8.06±0 ^{cd}	0.43±0.0	3.36±0 ^{cde}	0.87± 02 ^{ab}	0.095±0^{abc}	0.2±0 ^{b}	95.97±0.01
		Min	8.06	0.43	3.36	0.86	0.095	0.21	95.9
		max	8.06	0.43	3.36	0.86	0.095	0.21	95.94
	Chagni	mean±std	7.14±0.13 ^{cd}	0.45±0.001	2.99±0.04 ^{def}	0.87±0.01 ^{abc}	0.08±0.0 ^{bc}	0.25±0.026 ^{b}	87.51±2.0
		Min	7.05	0.4	2.96	0.86	0.08	0.23	86.00
		max	7.24	0.5	3.06	0.87	0.08	0.26	88.94
	Ayehu Guagus	mean±std	7.02±0.26 ^{cd}	0.38±0.017	2.8±0.12 ^{ef}	0.79±0.08 ^{abc}	0.08±0.004 ^{bc}	0.24±0.047 ^{b}	85.22±3.06
		Min	6.73	0.36	2.68	0.68	0.076	0.17	82.16
		max	7.33	0.397	2.94	0.87	0.085	0.28	89.03
	Banja	mean±std	9.83±0.03 ^{abc}	0.45±0.002	4.30±0.22 ^{abc}	1.23±0.05 ^{ab}	0.1±0.004 ^{bc}	0.32±0.002^{b}	98.57±1.31
		Min	9.8	0.45	4.14	1.197	0.1	0.31	92.18
		max	9.85	0.46	4.46	1.27	0.12	0.32	95.78
East Gojjam zone	Basolibien	mean±std	7.44±0.65 ^{cd}	0.37±0.05	2.894±0.17 ^{ef}	0.776±0.09 ^{abc}	0.08±0.009 ^{bc}	0.21±0.02 ^{b}	83.73±7.49
		Min	6.78	0.29	2.65	0.57	0.07	0.19	72.63
		max	8.568	0.46	3.18	0.87	0.09	0.25	95.24
	Degen	mean±std	6.65±1.37 ^{d}	0.3±0.19	2.3±0.73 ^{ef}	0.65±0.18 ^{bc}	0.067±0.01 ^{c}	0.165±0.07 ^{b}	78.45±7.85
		Min	5.34	0.04	1.439	0.46	0.05	0.07	69.91
		max	8.59	0.47	3.1	0.87	0.09	0.22	86.92
	Deber Elias	mean±std	7.39±1.0 ^{cd}	0.41±0.06	3.02±0.52 ^{f}	0.75±0.18 ^{abc}	0.074±0.008 ^{bc}	0.26±0.05 ^{b}	84.70±9.34
		Min	6.50	0.37	2.6	0.55	0.07	0.21	76.75
		max	8.77	0.51	3.74	0.96	0.09	0.34	96.92
	Gozamen	mean±std	7.56±1.32 ^{cd}	0.44±0.09	3.10±0.23 ^{def}	0.76±0.06^{abc}	0.09±0.001 ^{bc}	0.22±0.002 ^{b}	92.52±16.0
		Min	6.2	0.32	2.8	0.7	0.07	0.19	77.34
		max	8.7	0.51	3.3	0.83	0.1	0.2	107.15
		p	*	**ns**	*	*	*	*	

P=probability level, significance at p<0.05 and ns = not significant at p>0.05.Means followed by the same letters in a column are not a significant difference (p<0.05).*

4.3.1. Fatty acid profile of coffee beans in Four Woredas of West Gojjam Zone

The results of the analysis showed that the fatty acid (419.5 mg g^{-1}) contents of woredas found in West Gojjam zone ranged from 93.90 (Finoteselam) to 123.54 mg g^{-1} (Bure city administration) with a mean value of 102.74 mg g^{-1} (**Table 8**). The analysis of variance showed that there was significant difference ($p<0.05$) between woredas found in West Gojjam zone in the fatty acid contents of palmitic acid, linolic acid, oleic acid, stearic acid and arachidic acids whereas, the rest of nine fatty acids were not significantly different ($p>0.05$).

Coffee beans from Bure city administration were found to contain significantly higher concentration of total fatty acid (123.54 mg g^{-1}) than in those from other three woredas, namely, Finoteselam, Bure Zuria woreda and JabieTehnan woreda with average fatty acid concentrations of 93.90, 97.72 and 104.88 mg g^{-1} respectively of coffee beans.

From the major fatty acid, palmitic (39.97 mg g^{-1}) and linoleic acid (46.64 mg g^{-1}) were significantly higher in samples from Bure city administration than in those from Bure Zuria woreda (34.55 and 37.40 mg g^{-1} respectively). Similarly, the concentration of oleic acid (15.0), stearic acid (12.43 mg g^{-1}) and arachidic acid (4.90 mg g^{-1}) were present in the sample from Bure city administration significantly higher than from Finotselam coffee beans (8.94, 7.75 and 3.08 mg g^{-1} respectively).

4.3.2. Fatty acid profile of coffee beans in Four Woredas of East Gojjam Zones

The fatty acid (339.35 mg g^{-1}) contents of woredas found in East Gojjam zone ranged from 78.45 (Dejen Woreda) to 92.52 mg g^{-1} (Gozamen woreda) with the mean value 84.56 mg g^{-1} (**Table 8**). The analysis of variance showed that there was significant difference ($p<0.05$) between woredas found in East Gojjam zone in the fatty acid contents of pentadecanoic acid, arachidic acid, gondoic acid, tricosanoic acid and lignoceric acid whereas, the rest of thirteen fatty acids fatty acids were not significance difference ($p>0.05$).

Coffee beans from Gozamen woreda were found to contain a significantly higher concentration of total fatty acid (92.52 mg g^{-1}) than in those from other three woredas,

namely, Dejen, Basoliben, and Debre Elias woredas with mean value fatty acid concentrations of 78.45, 83.73 and 84.70 mg g^{-1} coffee beans respectively.

From the minor fatty acids, pentadecanoic acid (0.15 mg g^{-1}) and tricosanoic acid (0.09 mg g^{-1}) were significantly higher in green coffee beans from Gozamen woreda than in those from Dejen woreda green coffee beans (0.14 and 0.067 mg g^{-1} respectively). In addition, the level of arachidic acid was significantly higher in Gozamen woreda green coffee beans (3.10 mg g^{-1}) than from Dejen woreda (2.3 mg g^{-1}) coffee bean samples. On the other hand, the concentration of lignoceric acid (0.26 mg g^{-1}) was significantly higher in the coffee sample from Debre Elias woreda than from Dejen woreda coffee bean samples with mean values (0.165 mg g^{-1}).

4.3.3. Fatty acid profile of coffee beans in Four Woredas of Awi zone

The total fatty acid (367.27 mg g^{-1}) contents of woredas found in Awi zone ranged from 85.22 (Ayehu Guagusa woreda) to 98.57 (Banja woreda) with a mean value of 90.55 mg g^{-1} (**Table 8**). The analysis of variance showed that there was a significant difference ($p<0.05$) between Woredas found in Awi zone in the fatty acid contents.

Coffee beans from Guanghua woreda and Banja woreda were found to contain a significantly higher concentration of total fatty acid (95.97 and 98.55 mg g^{-1}) than those from other two Woredas, namely Ayehu Guagusa and Chagni, with mean value fatty acid concentrations of (85.22 and 87.51 mg g^{-1}) Coffee beans respectively.

Among the major fatty acids, palmitic acid (34.82 mg g^{-1}), stearic acid (9.83 mg g^{-1}) and arachidic acid (4.30 mg g^{-1}) found in green coffee beans in Banja woreda were significantly higher than the rest woredas. Likewise, from the minor level of tricosanoic, Myristric and gondoic acid (0.10, 0.148 and 0.45 mg g^{-1}) respectively were significantly higher than the coffee sample of the rest woredas. In addition, the concentration of linoleic acid present in samples from Guangua (39.18 mg g^{-1}) was significantly higher than the rest of woredas. Similarly, oleic acid (10.7 mg g^{-1}) found in green coffee beans from Guangua woreda was significantly higher than the rset of werdas. On the other hand, from the minor concentration of fatty acids, margaric acid (0.185 mg g^{-1}) was found in green coffee beans in Guanghua woreda significantly higher than the rest of woredas green coffee beans.

4.4. Comparison of Fatty Acid constituents of Green Coffee Beans with Reported Values

The average concentration corresponding to each of the thirteen fatty acids determined in the three zones samples were compared with some reported fatty acids in green coffee beans from other countries (**Table 9**).

The levels of palmitic acid found in this study were ranged from (32.17-38.52%) with a mean value of 35.35 %. The result of this study was in agreements with (Joet *et al.,* 2010) who reported that 35% of palmitic acid in the coffee bean of Reunion Island. Likewise, (Villarreal *et al.,* 2009) also reported that the coffee bean of Colombia contained 32-35 % of palmitic acids. In contrary, the palmitic acid content of this study was found above the coffee bean of Brazil which was 26.6-27.8 % (Speer, 1993).

The concentration of linoleic acid presented in this study was ranged from 36.97-42.49 % with mean values of 39.73%. The results of this study were in agreements with (Villarreal *et al.,* 2009) who reported 41-46 % of linoleic acid in green coffee beans collected from different geographical origins of Colombia. Similarly, (Joet *et al.,* 2010) reported that coffee beans of Reunion Island were contained 44% of linoleic acid. However, the concentration of linoleic acid of this study was below the coffee beans of Brazil which was 52.2-54.3% (Speer, 1993).

The contents of gondoic acid (0. 5-0.55%) determined in this study were in agreement with the concentrations of coffee beans (0.3%) reported from Reunion Island. However, it is five times lower than (Bertrand *et al.,* 2008) the values of (2.5−3.0%) reported in Colombian coffee beans. In addition, the level of lignoceric acid found in this study was ranged from 0.9-2.56%, which was eight times greater than (Bertrand *et al.,* 2008) the values (0.23−0.3%) reported in coffee beans from Colombia.

The concentration of oleic acid (5.9−14.34%), behenic acid (0.1−1.4%) and stearic acid (7.45−14.41%) were found in this study of green coffee beans. Those results were in agreements with the result (Bertrand *et al*., 2008) of coffee beans reported from Colombia. Likewise, (Joet *et al.,* 2010) also reported that the coffee bean of Reunion Island was contained 7, 0.5, and 7% of oleic acid, behenic acid, and stearic acid respectively.

Table 9: Comparison of the concentrations (percentage by weight of total fatty acids) of individual fatty acids determined in green coffee beans of the Arabica variety from different countries.

	Sample Origin					
Fatty Acids	Reunion Island(mg g^{-1})	Colombia (mg g^{-1})	Colombia (mg g^{-1})	Unspecified (mg g^{-1})	Brazil (mg g^{-1})	The present Study (mg g^{-1})
Myristric Acid	-	-	-	0.07-0.09	-	0.12-0.2
Palmitic Acid	35±1	32-35	33-36	33-36	26.6-27.8	32.17-38.52
Margaric Acid	-	0.1-0.11	-	-	-	0.13-2.85
Linoleic Acid	44±2	44-45	41-46	43-46	52.2-54.3	36.97-42.49
Oleic Acid	7±1	9-10	8-12	7-9	6.7-8.2	5.95-14.34
Stearic Acid	7±1	7-8	9-10	7-9	5.6-6.3	7.45-14.41
Gondoic Acid	0.3±0.1	2.5-3	-	0.31-0.36	-	0. 55-0.55
Arachidic Acid	2.4±0.5	0.33-0.36	22-3.0	2.5-3.4	2.6-2.8	1.97-4.49
Behenic Acid	0.5±0,1	0.6-0. 8	0.5-0.8	0.4-0.9	0.5-0.6	0.03-1.14
Lignoceric Acid	0.2±0.1	0.23-0.30	-	-	0.2-0.4	0.09-2.56
Reference	Joet *et al*. (2010)	Bertrand *et al*. (2008	Villarreal *et al* (2009)	Martin *et al.* (2001)	Speer (1993**)**	This study

The observation of variation in fatty acid concentration between reported values of coffee beans and the three zones of Amhara region's coffee might be due to the differences in the genetic properties and environmental growing conditions of the coffee growing areas as well as harvesting, processing and storage conditions. It has been proved that as the coffee beans are stored for extended period of time, the fatty acid content increases drastically. A report from Brazil shown that 10-year-old coffee contained more than 30 $g.kg^{-1}$ of total fatty acid which was only one g kg-1 during the harvesting time (Speer *et al*., 2004).

5. CONCLUSION AND RECOMMENDATION

5.2. Conclusion

In this study, the variation of coffee samples at zonal and woreda level, based on fatty acid profiles of coffee in Amhara region, was demonstrated. A total of twenty three fatty acids, sixteen saturated and seven unsaturated fatty acids, were detected in all of the green coffee bean samples using GC-MS: Among these myristic (C14:0), pentadecanoic acid(C15:0), palmitic (C16:0), margaric acid (C17:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), arachidic (C20:0), gondoic acid (C20:1), 14-methyleicosanoic acid (C21:0), behenic acid (C22:0), tricosanoic acid (C23:0) and lignoceric acid (C24:0) were the major ones. There was significant difference ($p<0.05$) between zones and woredas in the concentration of total fatty acids. The total fatty acid content of West Gojjam zone (102. 74 mg g^{-1}) was significantly higher than East Gojjam (84.56 mg g^{-1}) and Awi (90.55 mg g^{-1}) zones. Bure city administration coffee beans from West Gojjam zone (123.54 mg g^{-1}) content of total fatty acids significantly higher than the rest of twelve woredas coffee types, which were ranged from (78.45-104.88 mg g^{-1}). Myristric, palmitic, linoleic, stearic, arachidic and tricosanoic acids were identified as the most important fatty acids for variation of coffee samples studied in this region. Among the determined fatty acids, linoleic acid was found to be the major fatty acid in all zones. The fatty acid content of the coffee samples of the current study was found to comparable with the previously reported literature values.

5.2. Recommendation

The fatty acid profile of this study may be taken as one possible tool for authentication of the region's coffee.

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