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Identification and Determination of Fatty Acids In Green Coffee Beans and Correlating With Their Cup-Quality: The Case of Awi Zone, Zegie and South Gondar Zone of Amhara Region, Ethiopia

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IDENTIFICATION AND DETERMINATION OF FATTY ACIDS IN GREEN COFFEE BEANS AND CORRELATING WITH THEIR CUP-QUALITY: THE CASE OF AWI ZONE, ZEGIE AND SOUTH GONDAR ZONE OF AMHARA REGION, 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 is titled "IDENTIFICATION AND DETERMINATION OF FATTY ACIDS IN GREEN COFFEE BEANS AND CORRELATION WITH THEIR CUP-QUALITY: THE CASE OF AWI ZONE, ZEGIE AND SOUTH GONDAR ZONE OF AMHARA REGION."

Awoke Gashaw is approved for the degree of Master of Sciences in Analytical Chemistry.

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DECLARATION

This is to certify that the thesis entitled "IDENTIFICATION AND DETERMINATION OF FATTY ACIDS IN GREEN COFFEE BEANS AND CORRELATING WITH THEIR CUP-QUALITY: THE CASE OF AWI ZONE, ZEGIE AND SOUTH GONDAR ZONE OF AMHARA REGION" is submitted in partial fulfillment of the requirements for the degree of Master of Science in Analytical Chemistry to the post-Graduate Program of the College of Science, Bahir Dar University is an authentic work conducted by Awoke Gashaw Abate (PhD).

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ABBREVIATION

FA_S	Fatty Acid
ECX	Ethiopian Commodity Exchange
GD	Growth Development
$PUFA_S$	Polyunsaturated Fatty Acids
FFA	Free Fatty Acid
RH	Relative humidity
AFA	Peak Area Of The Fatty Acids
SPSS	Stastical Package Of Social Science
ANOVA	One Way Analysis Of Variance
RT	Retention Time
MUFA	Monounsaturated Fatty Acid

ABSTRACT

The green coffee samples were collected from three zones in the Amhara region, assigned to ensure representation of the producing areas, namely the Awi zone, South Gondar zone, and Zegie peninsulae. A total of forty-four samples were collected. Of these, thirty samples from the Awi zone, nine samples from the Bahir Dar Administrative zone and five samples from the South Gondar Zone were collected during the 2021 crop season using a judgmental sampling technique. The Folch method was used to extract lipids from green coffee beans. 0.500 g of coffee powder was extracted with chloroform and methanol (2:1 ratio v/v) by shaking at 270 rpm for 24 hours on a platform shaker with the aid of a test tube. A total of twelve fatty acids, eight saturated and four unsaturated fatty acids were detected in all green coffee bean samples. The highest fatty acid content of green coffee beans in three zones were myristic, pentadecanoic ,palmitic, oleic ,linoleic, arachidic, gondoic, behenic, tricosanoic, and lignoceric acid. Linoleic acid was the most abundant fatty acid in the green coffee beans and accounted for 44.27–47.31% of the total fatty acid content. The level of palmitic acid found in this study ranged from 42.87% to 53.47%.

Keywords: *Green Coffee Beans; Fatty acids; Gas Chromatography Mass Spectrometry*

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Green coffee is one of the most traded agricultural commodities in the world (Hung, Chen, & Chen, 2018). Due to its pleasant taste, scent, stimulating impact, and health advantages, coffee is the most widely consumed beverage in the world. The word "coffee" originates from where Ethiopian shepherds discovered coffee beans in the sixth century (Gebrekidan et al., 2019). Coffee's popularity arises from its pleasant taste sensation, psychostimulant effects, and enticing health benefits (Zhu, Long, Ma, et al., 2021). There are over 100 varieties of coffee, but only two are commercially grown: Coffea arabica L (Arabica coffee) and Coffea canephora (Robusta coffee), which account for 60% and 40% of world coffee consumption, respectively (Scholz et al., 2016).

Arabica coffee is preferred over Robusta coffee because of its lower bitterness and better aroma characteristics. Arabica coffee accounts for over 95% of world coffee production (Zhu, Long, Ma, et al., 2021). It is still the only one grown in Ethiopia, but an impressive selection of different Arabica types with characteristic flavors, depending on their geographical origin, are produced and have become popular in many parts of the world (Mehari et al., 2019). Each coffee species' quality differs depending on its cultivars, geographical origin, and environmental factors including altitude, temperature, and soil (Dong, Tan, Zhao, Hu, & Lu, 2015).

Fatty acids (FAs) are a type of lipid found in nature, food, and organisms and are a key component of cell membranes. They have important biological, structural, and functional roles, and a significant source of energy(Nagy & Tiuca, 2017). Fatty acids are an important criterion for determining the quality of coffee(Tsegay et al., 2020). Genetic features, agronomic practices, harvesting, postharvest circumstances, and environmental factors all influence the fatty acid profiles of green coffee beans. The amounts of fatty acids can be used to determine the geographical origin of green coffee beans because these parameters differ from one location to the next (Mehari et al., 2019).

Some studies examine identifying and determining fatty acids in green coffee beans collected from different geographical origins. These reports noted that the individual fatty acid compositions vary with respect to the coffee's region of origin. Ethiopia is one of the most coffee-producing countries in the world. However, most researchers in this field do not look at fatty acids in green coffee beans collected from the Amhara region. In addition, the total lipid as have not been correlated with the cup-quality parameters of the region's coffee. Therefore, the primary goal of this study was to identify and quantify the fatty acid content of green coffee beans collected from the Awi zone, South Gondar Zone, and Zegie peninsula of the Amhara region and to correlate it with their cup-quality parameters.

1.2 STATEMENT OF THE PROBLEM

Coffee is the world's second most traded raw material. The most important source of foreign exchange for many agriculturally oriented countries, an attractive source of tax revenue, and the most popular beverage. Due to the economic importance of coffee, there is an increasing demand for proper quality control for certification of contents and substandard products (Weldegebreal, Redi-Abshiro, & Chandravanshi, 2017). Due to the economic importance of coffee, there is an increasing demand for proper quality control for certification of contents and substandard products (Weldegebreal et al., 2017). The quality of green coffee beans is largely determined by the altitude and soil characteristics at which the plant is grown. Linoleic (C18:2), palmitic (C16:0), oleic (C18:1), arachidic (C20:0), and linolenic acids are the primary fatty acids found in green coffee beans. Linoleic and palmitic acids are the most abundant fatty acids in green coffee beans (Tsegay et al., 2020). Although coffee is grown in areas in the Amhara region, its fatty acid content has yet to be studied spatiotemporally. As a result, this study aims to identify and determine the content of fatty acids in green coffee beans cultivated in selected districts in the Amhara region and compare the total lipids with the coffee's cup quality tests.

1.3 GENERAL OBJECTIVE OF THE STUDY

The general objective of the study was to identify and determine fatty acids in green coffee beans of the three zones by using GC-MS and correlating them with their cup quality.

1.3.1 SPECIFIC OBJECTIVE

- ✓ To identify the avilable fatty acids in the green coffee samples.
- ✓ To quantify the identified fatty acids in the green coffee samples.

- ✓ To compare the total and individual fatty acids between coffee of the three sampling zone, (Awi Zone,South Gonder and Bahir Dar special Zone).
- ✓ To correlate the fatty acids composition with the coffee cup quality test.
- ✓ To compare the fatty acid composition of the study area with reported values from elsewhere.

1.4. SCOPE OF THE STUDY

This study focused on identifying and determining fatty acids in green coffee beans grown in selected areas in three zones of the Amhara region (Awi, Bahir Dar special zone, and South Gondar Zone).

1.5. SIGNIFICANCE OF THE STUDY

The study will support the continued effort to brand the region's coffee and enhance its market share at ECX.

CHAPTER TWO

LETERATURE REVIEW

2.1 HISTORY OF GREEN COFFEE BEANS

The word "coffee" comes from the province of Kaffa, where shepherds from Abyssinia/Ethiopia discovered the coffee beans in the sixth century (Hagos et al., 2018). Coffee has undergone various physical transformations throughout history, beginning as an energy source for nomadic cultures who mixed coffee berries with animal fat to create an early type of energy bar. Later, it was used as tea, then as wine, and lastly as the beverage we know today (Maurya, 2016).

More than 1,000 years ago, a gatherd in Ethiopia's southwestern highlands selected a few red berries from some young green trees growing in the forest and sampled them to see if they had flavor and gave the customers a good feeling (Amamo, 2014).

Ethiopians have a long history with coffee, and coffee production and consumption are inextricably linked to Ethiopian history, culture, and economy. Coffee has been grown, traded, and consumed for centuries, and it continues to play an important role in the everyday lives of most Ethiopians and the state as a whole (Bossolasco, 2009).

Coffea arabica and Coffea canephora (commonly known as "robusta") are the two most common commercial coffee bean varieties, which differ in the chemical content of the green coffee bean. Ethiopia is the birthplace of Arabica coffee (Coffea arabica L) (Alemayehu & Merga, 2017). Arabica coffee is higher in lipids, but robusta coffee is higher in caffeine and polyphenols (Godos et al., 2014). Arabica coffee beans are the most valuable in the international commerce (80%), as they are thought to have a better flavor than robusta (Godos et al., 2014). Arabica coffee originated in Ethiopia and moved to India, Indonesia, Brazil, Colombia, and Central America (Buffo & Cardelli-Freire, 2004).

Coffee is grown at various altitudes in Ethiopia, ranging from 550 to 2,750 meters above sea level. On the other hand, Arabica coffee thrives and produces best between 1,300 and 1,800 meters, with annual rainfall ranging from 1,500 to 2,500 mm and appropriate minimum and

maximum air temperatures of 15 and 30 degrees Celsius, respectively (Tadesse, Tesfaye, & Abera, 2020).

2.2 COFFEE PRODUCING COUNTRY IN THE WORLD

Coffee is one of the world's most important non-alcoholic beverage crops, farmed in over 80 countries in the tropical and subtropical areas of the world, exported in various forms to over 165 countries, and providing a livelihood for over 25 million coffee-farming families (Kathurima, Gichimu, Kenji, Muhoho, & Boulanger, 2009). For various developing countries in Africa, Asia, and Latin America, including Ethiopia, coffee is a key source of income (Wale Mengistu, Alemayehu Workie, & Sualeh Mohammed, 2020). Brazil is now the world's largest producer of coffee.Vietnam is in second place, with Colombia trailing well behind in third (Mehari et al., 2019). Arabica coffee is grown in Costa Rica, Guatemala, Colombia, Brazil, Kenya, Ethiopia, Sumatra, and Sulawesi, among other places (Joët et al., 2010).

Ethiopia is Africa's leading coffee producer, ranking fifth in the world behind Brazil, Vietnam, Colombia, and Indonesia (Wale Mengistu et al., 2020). Coffee is grown practically everywhere in Ethiopia.There are 600,000 hectares of coffee-growing land. Ethiopia's main coffee-growing regions include the west and south west, the southern, eastern, and central regions (Degaga, 2020). Kaffa, Illubabor, Wollega, Gimbi, Sidamo, Gedeo, (including) YirgaCheffe, and Harrarge are the important locations so far (Wale Mengistu et al., 2020).

According to the Central Statistical Agency, Ethiopian coffee production is increasing, with current production estimated to be 449,229.8 t on 725,961.2 hectares of land with average productivity of 0.612 t ha^{-1} clean coffee (CSA). Coffee production in the Amhara region is expected to be 3,006.8 t, with productivity of 0.302 t ha^{-1}(Wale Mengistu et al., 2020).

2.3 ARABICA COFFEE

Among other common type of coffees, coffee Arabica is the most common.The Arabica species produces all of the world's highest-graded coffee and beans with speciality rank. This type of coffee is one of the most essential and valuable commodities sold in the world (Cheng, Furtado, & Henry, 2018). Arabica coffee is noted for creating high-quality brews (Barbosa, dos Santos Scholz, Kitzberger, & de Toledo Benassi, 2019). The Coffea arabica plant, also known as arabica coffee, prefers humid regions and cannot withstand frost (Haines, 2019). The Arabica species

produces all of the world's highest-graded coffee and beans with speciality rank (Knopp, Bytof, & Selmar, 2006).

Arabica coffee is an important feature of Ethiopia's tropical forest agroecology in the southwest (Tassew, Yadessa, Bote, & Obso, 2022). Arabica coffees are thought to be of higher grade and have a more refined flavor profile than Robusta coffees (Keidel, von Stetten, Rodrigues, Máguas, & Hildebrandt, 2010). The origins of coffee can be traced back to Ethiopia, where the coffee plant, coffee arabica, still grows wild in the highlands' forests between altitudes of 1100 and 2200 meters, with annual rainfall ranging from 1500 to 2500 mm, in slightly acidic soil with a pH of 4.5-6.5 (Chauhan, Hooda, & Tanga, 2015).

Ethiopia has the greatest highland area suited for Arabica coffee production and hence has the potential to be a leading producer in terms of both quality and quantity. Arabica coffee contributes to the lion's share of foreign currency profits, accounting for up to 35% of total GD earnings (Adugna, 2021).

2.4 QUALITY OF GREEN COFFEE BEANS (ARABICA COFFEE)

Quality is a key feature of coffee, and it is becoming even more so as the world market becomes increasingly competitive (García, Candelo-Becerra, & Hoyos, 2019). The coffee industry places a premium on quality, which includes fragrance, aroma, flavor, sweetness, and acidity, as well as physical properties such as length, width, thickness, or weight, shape, and color of coffee beans (Wale Mengistu et al., 2020). Single origin coffee development is a crucial method for maintaining coffee quality, grade, and a high cupping score (Putri, Irifune, & Fukusaki, 2019).

The majority of data on coffee quality in Ethiopia comes from the Ethiopian Commodity Exchange (ECX) Company's assessment of green coffee beans as they arrive in the central market (Mintesnot, Dechassa, & Mohammed, 2015). Every day, more than two billion cups of coffee are consumed around the world (García et al., 2019). As a result, coffee consumption and demand for high-quality coffee beans have risen over time. Therefore, evaluating the quality of green coffee beans has become crucial for market pricing, storage stability, and customer acceptance (Haile & Kang, 2019).

2.5 CHEMICAL COMPOSITION OF GREEN COFFEE BEANS

Chemical components are the foundation of biological processes and give coffee its distinct aroma. Coffee is a complex mixture of over a thousand different compounds (Godos et al., 2014). Caffeine, tannin, fixed oil, carbohydrates, and proteins are the primary components of green coffee beans. In the present, the chemical composition of green coffee bean contents is caffeine 2-3%, tannins 3-5%, proteins 13%, and fixedoils 10-15% (Sharma, 2020).

Alkaloids, phenolic acids, flavonoids, terpenoids, and other chemical components are found in green coffee beans (Saud & Salamatullah, 2021). Chemically, coffee is mostly made up of carbohydrates, lipids, and polyphenols (Mehari et al., 2021). Phenolic chemicals and their derivatives (such as chlorogenic acids), alkaloids (including caffeine), diterpenoid alcohols (such as cafestol and kahweol), carbohydrates, lipids, and volatile and heterocyclic compounds are among the chemical elements of arabica coffee (Affonso et al., 2016).

The coffee bean's composition determines the coffee bean's quality (Cheng et al., 2018). The chemical composition of coffee beans varies depending on the species, height, soil, temperature fluctuations, and the location where it grows (Tsegay et al., 2019). The chemical composition of coffee beans, as well as the post-harvesting process, agricultural techniques, and agro-ecological factors, all influence its quality (Adamu, 2019). Genetic variation, post-harvest processing procedures, bean maturation, farming practices, and environmental gradients all influence the chemical makeup of green coffee beans (Bobková et al., 2022).

Table 1. shows the chemical composition of roasted Arabica and Robusta coffee seeds (Muzaifa, Hasni, Patria, & Abubakar, 2020).

Components	Concentration (g100 g-1)	
Carbohydrates	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
Lipida		
Coffee oil	15–17	7.0–10.0
Diterpenes(freeand 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

The information is shown above (Table 1) focuses on the primary elements of coffee beans, including caffeine, carbohydrates, chlorogenic acids, lipids, other nitrogenous chemicals, volatiles, and the processes that lead to these changes. The cultivar, farming methods, climate, soil composition, and analytical techniques all affect content differently.

2.6 THE GREEN COFFEE BEANS FACTORS

The quality of coffee is determined by a variety of things. The key factors that impact the overall quality of coffee produced in a given location are the coffee production and processing processes (Tassew et al., 2022). Environmental factors such as height, daily temperature changes, rainfall volume and distribution, and physical and chemical qualities of the soils all influence coffee quality (Wale Mengistu et al., 2020). Raw bean size , shape, and color are used to determine coffee quality. Organoleptic methods are used to determine cup quality (Njoroge, 1998). The sensory qualities and quantities of coffee beverages are determined by the production method (Baqueta, Coqueiro, Março, & Valderrama, 2020).

Agronomic factors such as edaphoclimatic conditions and coffee genetics have an impact on the composition of green coffee beans and, as a result, the coffee brew that results (Barbosa et al., 2019).

2.7 BIOLOGICAL USE OF COFFEE FOR HUMANE BEINGS

A lot of recent experimental and epidemiological research has found that coffee drinking has a significant favorable influence on human health (Godos et al., 2014). Coffee has a lot of different chemicals in it, including antioxidants, chlorogenic acids, etc. Worldwide, coffee is the most common beverage after water, with approximately 500 billion cups consumed annually, and this number continues to increase (Parikh, 2013). With such widespread consumption, it becomes important to consider the effects of coffee on our health. Coffee may prevent type 2 diabetes and neurological disorders like Alzheimer's, according to new research. Coffee's absorption and profile of both beneficial and toxic chemicals is complicated and dependent on a number of factors (Maurya, 2016).

2.8 LIPIDS

Lipids are the most important components of coffee beans. The lipid content consists of wax, triglycerides, and unsaponifiable matter; the oil content in *C. arabica* is about 16.0% (Wagemaker, Carvalho, Maia, Baggio, & Guerreiro Filho, 2011). Green coffee beans have a lipid content that ranges from 10% -17% ,(Luisa et al., 2015). The review of (Caporaso, Whitworth, Grebby, & Fisk, 2018),reported that Lipids are important component of green coffee beans, with lipid content ranging from 7-10% in Robusta coffee and up to 15-17% in Arabica. The results of this study were in agreement with (Luisa et al., 2015) and ranged from 10% to 17%. According to (Zhu, Long, Chen, et al., 2021), the total lipid content of green coffee beans in Indonesia, Colombia, and Brazil ranged from 12.88–13.62, 13.37–14.59, and 12.88–14.86, respectively.

Table 2**:** the ranges values of lipid content in green coffee beans (dry weight) from different geographical origins (Zhu, Long, Chen, et al., 2021).

Geographical origins	Lipids (g 100 g− 1)
China	14.75− 15.67
Indonesia	12.88− 13.62
enya	14.14− 16.15
Ethiopia	14.33− 15.19
Guat mala	15.23− 16.29
Honduras	14.05− 15.53
Colombia	13.37− 14.59
Brazil	12.88− 14.86

2.8 FATTY ACIDS IN GREEN COFFEE BEANS

Fatty acids are long-chain hydrocarbons that can be divided into four categories: saturated, monounsaturated, polyunsaturated, and trans fatty acids. Each carbon in saturated fatty acids is bonded to two hydrogen atoms, with single bonds between the carbons. *Unsaturated fatty acids* have one or more points of unsaturation, or double bonds between the carbon atoms (Wanders et al., 2017). Saturated and trans fatty acids have been linked to an increased risk of coronary heart disease. Monounsaturated and polyunsaturated fatty acids have been linked to a lower risk of coronary heart disease (White, 2009).

Coffee's lipid fraction is mostly made up of triacylglycerols, sterols, and tocopherols, which are common components in all edible vegetable oils (Speer & Kölling-Speer, 2006). Triacylglycerols with fatty acids make up the majority of coffee oil (Speer & Kölling-Speer, 2006). Fatty acids are not found in nature in a free state; instead, they are typically found in the form of triglycerides, which are fatty acids combined with glycerol (alcohol)(Gunstone, 2012). The oil in the endosperm of the bean contains the most lipid portion in green coffee. The bean's outer layers also contain a minor quantity of wax (Mehari et al., 2019).

Saturated and unsaturated fatty acids are distinguished by the presence or absence of a double bond in the fatty acid chain (Kanchanamayoon & Kanenil, 2007). Oleic acid (C18:1), linoleic acid with two double bonds (C18:2), linolenic acid with three double bonds (C18:3), and, arachidonic acid, which contains four double bonds (C20:4), are all examples of unsaturated

fatty acids. Unsaturated fatty acids such as oleic (18:1n-9), linoleic (18:2n-6), and linolenic (18:2n-6) are abundant in green coffee beans (Dong et al., 2015). Linoleic (C18:2), palmitic (C16:0), stearic (C18:0), oleic (C18:1), arachidic (C20:0), and linolenic acids are the primary fatty acids found in green coffee beans. Linoleic and palmitic acids are the most abundant fatty acids in green coffee beans (Tsegay et al., 2020).

The term "essential fatty acid" refers to a fatty acid that the body cannot produce and must acquire from food. Dietary sources of essential fatty acids (linoleic and a-linolenic) are required (Glick & Fischer, 2013). Polyunsaturated fatty acids (PUFAs) are especially significant since they can help prevent cardiovascular disease, psychological problems, and a variety of other ailments like atherosclerosis, thrombogenesis, high blood pressure, cancer, and skin diseases (Robert, Mfilinge, Limbu, & Mwita, 2014). The fact that the two polyunsaturated fatty acids, linoleic and linolenic acids, are essential and unobtainable in the human body unless through dietary means emphasizes the importance of unsaturated fatty acids. Linoleic acid, an unsaturated omega-6 fatty acid present in a variety of plants, including pumpkin seeds, canola oil, soy beans, and flaxseeds, plays a critical role in a variety of human biological functions, including the neurological, skeletal, and reproductive systems, allowing them to work properly (Doan et al., 2019).

Fruits, vegetable oils, seeds, nuts, animal fats, and fish oils are all good sources of fatty acids (White, 2009). Many plant foods, such as safflower, sunflower, soybean, pine nuts, pecans, Brazil nuts, and corn oils, contain the fatty acid.

Fruits, vegetable oils, seeds, nuts, animal fats, and fish oils are all good sources of fatty acids (White, 2009). Many plant foods contain fatty acids such as safflower, sunflower, soybeans, pine nuts, pecans, Brazil nuts, and corn oils (White, 2009). Many plant foods contain fatty acids such as safflower, sunflower, soybean, pine nuts, pecans, Brazil nuts, and corn oils.

One of the main factors affecting coffee quality is the fatty acids found in green coffee beans. The coffee’s fatty acid makeup and the coffee plants' altitude both have an impact (Tsegay et al., 2020). Fatty acids are a significant criterion for determining coffee quality. Altitude, soil, and location are significant factors affecting the level of fatty acids in coffee. Fatty acids are known to be important components of coffee flavor and aroma.

Green Arabica coffee beans have an average lipid content of 15%, while Robusta coffees have less than 10% (Speer & Kölling-Speer, 2006). According to (Toci, Neto, Torres, & Farah, 2013), lipids are one of the major coffee components and correspond to approximately 11–20 g/100 g of roasted arabica Coffea arabica composition. Triacylglycerols (TAG) are the primary lipid class in coffee and make up 8–17 g/100 g in freshly brewed coffee (Toci et al., 2013).

According to (Muzaifa et al., 2020) green coffee bean samples' total lipid content ranges from 12.03 to 12.63 percent. According to a prior analysis, the lipid content of kopi luwak is between 12 to 18 percent, which is in the same range as standard Arabica coffee. Coffee's lipid components, also known as coffee oil, are broken down into linoleic acid (40-45%), palmitic acid-containing tryglycerides, and the oil that coats the surface of the bean (25-35%). Free fatty acids, diterpene alcohols, sterols, and tocopherols are the other lipid components and are typically present in edible vegetable oils. Other minor fatty acids, such as myristic acid (C14:0), margaric acid (C17:0), gondoic acid (C20:1), behenic acid (C22:0), and lignoceric acid (C24:0), were discovered at percentages of less than 1% (Zhu et al., 2021). Linoleic acid (43.58–44.02%) was the most abundant fatty acid in green coffee beans, followed by palmitic (35.42–35.64%), oleic (7.21–7.44%), stearic (7.02–7.09%), arachidic (2.36–2.37%), and linolenic acid (1.88–1.90%) (Zhu, Long, Ma, et al., 2021).

Table 3: Fatty acids in green coffee bean triacylglycerol (%) (Speer & Kölling-Speer, 2006).

Name of fatty acid	Folstar(1975)from 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	Trace	Trace
C24:0	Trace	0.3-0.4	0.2-0.4

Table 4: Fatty acid compassion (%) in green coffee beans in different countries (TIBEBU, 2018)

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

2.9 PRINCIPLE OF GC-MS AND ITS USE FOR FATTY ACIDS

GC/MS has long been used for the selective analysis of non-polar compounds. The carboxylic fatty acids require prior hydrolysis from their glycerolipid sources and derivatization to a respective ester form for separation on capillary chromatographic columns. The detection of structural molecular ions generated from the MS source provides more sensitive and selective assay of varied arrays of fatty acids present in lipid samples. Fast quadrapole technology present in most modern mass spectrometers facilitate for selective ion monitoring with simultaneous full scan capabilities (Johnston & Sobhi, 2017).

2.10. CUP QUALITY ANALYSIS

The assessment of coffee quality is a key step in price setting and determines its export potential in coffee producing countries. Consequently, it is of major importance to many coffee roasters and distributors. Cup quality characteristics are attributes of coffee that can be distinguished by the senses and can be assessed organoleptically by professional coffee tasters, based on established terminologies for cup quality analysis (e.g., flavor, acidity, body, and cup cleanness) (Barbosa et al., 2019). The attributes evaluated in the beverage quality, such as aroma, flavor, acidity, body, balance, preference, and sanitary quality of the bean, are important for the acceptance and definition of the bean quality (Luna González, Macías Lopez, Taboada Gaytan, & Morales Ramos, 2019).

CHAPTER THREE

METHODOLOGY

3.1. DESCRIPITION OF THE STUDY AREA

The study was carried out in Ethiopia's Amhara region. Especially the Awi Zone, Zegie, and South Gondar Zone.

3.1.1 AWI ZONE

. Agew Awi is bordered on the west by the Benishangul-Gumuz Region, on the north by the Semien Gondar Zone, and on the east by West Gojjam. It is named for the Awi sub-group of the Agaw people, some of whom live in this zone. The administrative center of Agew Awi is Injjbara. Other towns include Chagni, Agew, Gimiabet, Tilili, Adis Kidame Azena, Zigem, and Dangila.

Agaw Awi is relatively flat and fertile, whose elevations vary from 1,800 to 3,100m above sea level, with an average altitude of about 2,300m. The Zone is crossed by about nine permanent rivers which drain into the Abay (Blue Nile); other water features include two crater lakes, Zengena and Tirba, and Zimbiri marsh, which is located 5 km south-west of Addis Kidam. The Agaw have traditionally practiced a land management system that is well adapted to the local ecology, enabling them to sustain the soil fertility and minimize erosion; this area is recognized as one of the most productive in the Amhara Region. The farming system practiced in the study area is a mixed crop-livestock production system. the area has three defined seasons; dry season (October to January), short rainy season (February to May), and the long rainy season (June to September) (Abera, Mummed, Eshetu, Pilla, & Wondifraw, 2020).

3.1.2 SOUTHE GONDER ZONE (KORATA KEBELE)

South Gondar is a zone in the Ethiopian Amhara Region. This zone is named for the city of Gondar, the capital of Ethiopia until the mid-19th century, and has often been used as a name for the local province. South Gondar is bordered on the south by East Gojjam, on the southwest by West Gojjam and Bahir Dar, on the west by Lake Tana, on the north by North Gonder, on the northeast by Wag Hemra, and on the east by North Wollo; the Abby River separates South

Gondar from the two Gojjam zones. The highest point in South Gondar is Mount Guna (4231 meters) (Abera et al., 2020)

Elevation of scene topography varies from 1150 to 4225 meters above sea level (masl). It is roughly centered on Mount Guna in South Gondar and approximately half of the area included in the scene delivers water to the Blue Nile Basin. The scene includes the municipality of Debre Tabor, which lies at 2410 meters above sea level and experiences a highly seasonal monthly average rainfall ranging from 6 mm in January to 501 mm in July, and mean monthly temperature ranging from 15.0°C – 18.7°C (Min, 2016).

The maximum and minimum temperatures of the kebele are 29 oC and 23 oC, respectively, and the maximum and minimum rainfall are 1,000 mm and 750 mm, respectively (Tafere et al.).

3.1.3. BAHIR DAR ADMINISTRATIVE ZONE (ZEGIE ZURIA)

Bahir Dar Zuria is a woreda in the Amhara Region of Ethiopia, part of the West Gojjam Zone. This Woreda is bordered on the south by Yilmana Densa, on the northwest by Mecha, on the northwest by the Gilgel Abay River which separates it from Semien Achefer, on the north by Lake Tana, on the shores of which are situated the city and special zone of Bahir Dar, and on the east by the Abay River which separates it from the Debub Gonder Zone.

Bahir Dar Zuria includes the forested Zegie peninsula, known for its numerous medieval churches, of which the best known is Ura Kidane Mehret, and associated monasteries. Other points of interest include the Tis Issat falls and Dilde, better known as the Portuguese Bridge, over the Abay at Alata, about half a mile below the falls. The survey of the land in this woreda shows that 21% is arable or cultivable, 9% pasture, 8% forest or shrubland, 36% covered with water, and the remaining 26% is considered degraded or other. Teff, corn, sorgum, cotton, and sesame are important cash crops.

3.2 SAMPLING SITE AND SAMPLING TECHNIQUES

The green coffee samples were collected from three zones in the Amhara region, assigned to ensure representation of the producing areas, namely the Awi zone, South Gondar zone, and Zegie peninsulae. In each of the three sampling zones, purposively more productive wereda were considered. From the Awi zone, Ayehu Gagusa, Banja, Guangua, and Dangila Woredas, . From

the Bahir Dar administrative zone, Zegie Zuria woreda, and from the South Condar Zone, Dera Woreda were selected. From each wereda, samples were collected directly from the farmer. Three kilograms of sun-dried cherries were considered. The remaining 2 kg were submitted to ECX for cup-quality testing and the remaining 1 kg was taken for chemical analysis.

Accordingly, a total of forty-four samples were collected. Of these, thirty samples from the Awi zone, nine samples from the Bahir Dar Admnistrative zone (zegie zuria) and five samples from the South Gondar Zone were collected during the 2021 crop season using a judgmental sampling technique.

Once the coffee husk was removed, the green beans were subjected to room temperature drying just before grinding to fine powder by using an electronic grinder, sieved with 200-micron mesh. Then powder was immediately packed in a plastic cup with a stopper and kept at room temperature untile laboratory analysis.

Figure 1: Sample of green coffee beans

Table 2: The geographical zones, type, and assayed number of green coffee bean samples studied were:

Zone	Sample code	Woreda	Number of sample
Awi	45-66 and 66A-66H	Ayehu Gagusa	9
		Banja	3
		Guangua	9
		Dangila	9
South Gondar	76-80	Dera	5
Bahir Dar Admnistrative(Zegie zuria)	67-75	Bahir Dar Town	9

3. 3 CHEMICALS AND APPARATUS

3.3.1 APPARATUS AND EQUIPMENTE

Flatform shaker (ZHWY-334), centrifuge (model 800-1), plastic bag, GC-MS (Aglient Technologies 7890B-5977A, China), Electrical Girder (FW-100, High-Speed Universal Disintegrator girders), Oven (Universal hot air oven), Balance (electronic balance), Incubator (constant temperature and humidity incubator), Vacuum Rotary evaporator (RE100-pro), Refrigerator (digital inverter technology, Samsung), and syringe were used for laboratory analysis.

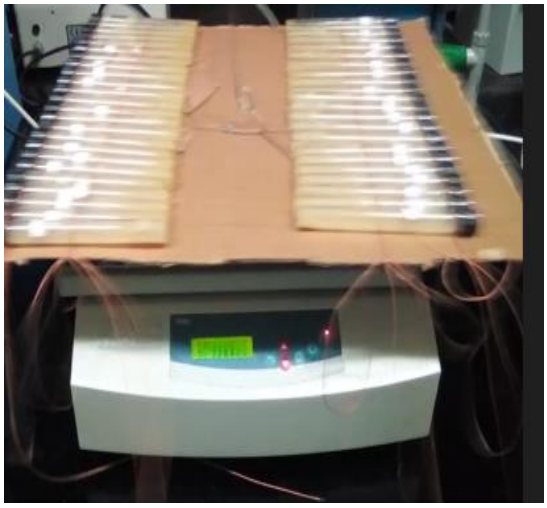
3.3.2 CHEMICALS

All reagents and standards used in the analysis were of analytical grade. Standard of fatty acids was used for the laboratory analysis. Other chemicals such as methanol absolute(Acetone Free-99.00%- solution , Alpha Chemika) , chloroform 99.8% (trichloromethane CHCl3) , toluene -99% (Blulux laboratory), n-hexane (99.9% -AR), acetone|(99.8 % , 400974- CARLO ERBA), sulfuric acid, anhydrous sodium sulfate, sodium chloride(99.5%,121001-Blulux laboratory),and distilled water was used for the laboratory analysis

3.4. SAMPLE PREPARATION FOR GC-MS ANALYSIS

3.4.1 EXTRACTION OF LIPIDS

The Folch method was used to extract lipids from green coffee beans (Breil, Abert Vian, Zemb, Kunz, & Chemat, 2017) . 0.500 g of coffee powder was extracted with chloroform and methanol (2:1 ratio v/v-1) by shaking at 270rpm for 24 hours on a platform shaker with the aid of a test tube. The filtrate was obtained after centrifuging the extract with the help of 2 ml of 0.73 percent aqueous sodium chloride. The lipid phase was separated, and then the upper phase was removed using a micropipette (siphoning), and the lower phase (chloroform) layer containing the lipid was recovered. The solvent was removed under a rotary vacuum evaporator, and the residue was washed with 5.0 mL of toluene.



A

B

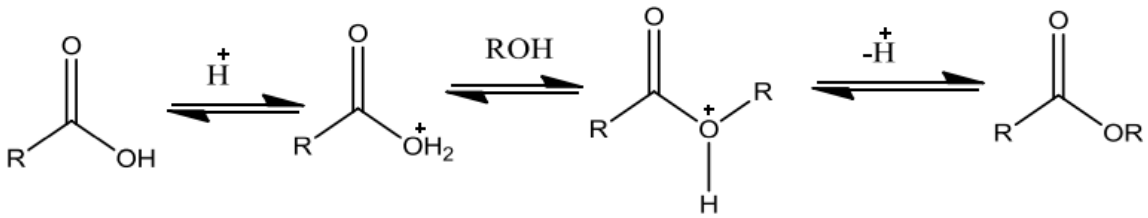
Figure 2**:** Extraction of lipids on platform shiker (A) and extracted lipids (B) from green coffee beans.

3.4.2 FATTY ACIDS DERIVATIZATION

Fatty acids are commonly derivatized to form fatty acid methyl esters (FAMEs), which are then identified using gas chromatography-mass spectrometry (Chiu & Kuo, 2020). The polarity and inadequate volatility of fatty acids make GC analysis difficult. The polar carboxyl groups must first be changed to more volatile non-polar derivatives before GC analysis can be performed. For

this purpose, variousalkylation reagents are available, and fatty acids are typically transformed into fatty acid methyl esters (FAMEs).

Briefly, a 1.0 mL sample of the lipid extract in toluene was spiked with 40 µL 0.6mg/ml nervonic acid and allowed to react for 12 hours with 2.0 mL of 1% methanolic sulfuric acid solution while being kept at 50°C in an incubator. After that, the reaction mixture was treated with 5.0 mL of a 5% aqueous sodium chloride solution and extracted twice with 5 mL of hexane. After phase separation, the upper phase was taken away, dried over anhydrous sodium sulfate, transferred using a micropipette, dried over anhydrous sodium sulfate, and then transferred to the vial for analysis by GC-MS. The following reaction is expected during the derivatization process (Christie, 1993).



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

3.5. CUP QUALITY ANALYSIS

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Cup quality is the summation of aromatic intensity, aromatic quality, acidity, astringency, bitterness, body, flavor, and overall standard (Wale Mengistu et al., 2020). Cup quality was tested by three experienced and certified professional panelists (cuppers). Each panelist gave

his/her independent judgment using a cupping form, where finally the average means of the records of the panelists were used.

3.6 GC-MS ANALYSIS

An Agilent Technologies 7890B gas chromatographic system equipped with an autosampler, a split splitless injector, and a mass spectrometer (Agilent Technologies 7890B-5977B) was used for GC-MS analysis of the fatty acid methyl esters. The following were the chromatographic conditions: 280°C injector temperature, DB-5 MS fused silica capillary column (30m x 250m x 0.25m), temperature programing settings of 40°C (held for 3 minutes), then ramped at 5°C min-1 to 230°C (held for 20 minutes), and helium as carrier gas at a flow rate of 1.68 ml min-1. The mass spectrometer (MS) was run with the following parameters: transfer temperature of 300 °C, scan range mz-1 of 40–600, ionization potential of 70 eV, and electron multiplier voltage of 3000 volts.

3.7 STATISTICAL ANALYSIS

Data analysis was performed using the statistical software package SPSS (IBM Corp, USA). Each data set consisted of a matrix in which the columns represented the individual green coffee bean samples and the rows consisted of the chromatographic peak areas of all the detected fatty acids or the concentrations of fatty acids determined . One way (ANOVA) was used to test the presence of significant differences in the mean content of fatty acids among the different zones of green coffee beans. At p 0.05, differences were considered significant. Whenever necessary, box plots (box and whisker plots) were contracted to display the differentiation among coffee types. Samples were extracted and analyzed in triplicate, and average values were used for statistical calculations.

CHAPTER FOUR

RESULTS AND DISCUSSION

A key factor in assessing the quality of coffee is the presence of lipids (Tsegay et al., 2020). The result of this study was that Arabica green coffee beans' average total lipid content was found to be 13.6244%, 13.1322%, and 12.6513%, respectively, in the Awi Zone, South Gondar Zone (korata), and Zegie. Green coffee beans from the Awi Zone are distinguished from those from other zones by their total lipid content. According to the results of this study (table 6), the mean values of total lipids found in Awi Zone green coffee beans (an average of 13.624%) are significantly higher than those found in beans from other zones. The maximum and minimum total lipid content values of the Awi zone were 19.9424% and 8.3325%, the South Gonder zone was 19.041% and 11.353%, and the Bhir Dar Zuria Zegie Pensiula was 17.1332% and 7.5826%, respectively.

Table 3**:** Statistical Distribution of Total Lipids Extracted from Green Coffee Beans in Awi Zone, South Gonder Zone,and Zegie Pensiula

Zones	N	Mean	Std.Deviation	Std.Error	Minimum	Maximum
Awi	30	13.62	3.099	1.386	8	20
South Gonder	5	13.13	3.592	0.656	11	19
Bahir Dar Zuria (Zegie)	9	12.65	3.067	1.022	8	17
Total	44	13.09	3.379	0.509	8	20

4.1. CUP QUALITY ANALYSIS

The assessment of coffee quality is a key step in price setting and determines its export potential in coffee producing countries. Consequently, it is of major importance to many coffee roasters and distributors. Cup quality characteristics are attributes of coffee that can be distinguished by the senses and can be assessed organoleptically by professional coffee tasters, based on established terminologies for cup quality analysis (e.g., flavor, acidity, body, and cup cleanness) (Barbosa et al., 2019). The attributes evaluated in the beverage quality, such as aroma, flavor, acidity, body, balance, preference, and sanitary quality of the bean, are important for the acceptance and definition of the bean quality (Luna González et al., 2019).

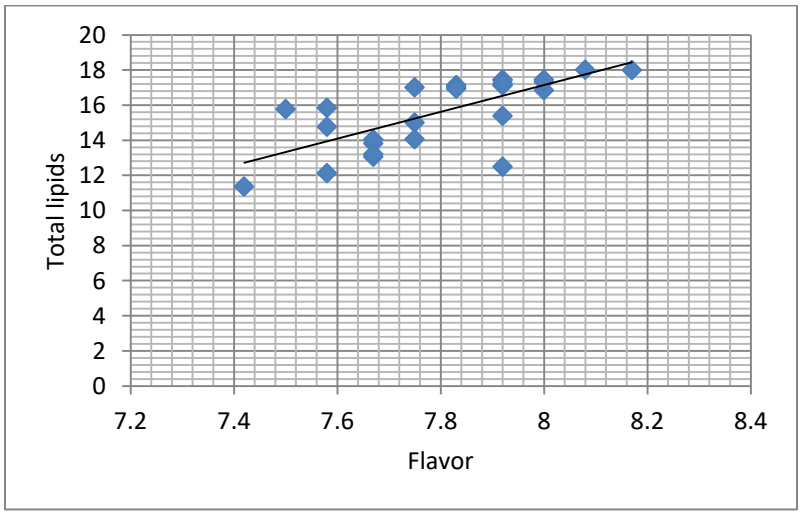
Cup quality is the summation of aromatic intensity, aromatic quality, acidity, astringency, bitterness, body, flavor, and overall standard (Wale Mengistu et al., 2020). Cup quality was tested by three experienced and certified professional panelists (cuppers). Each panelist gave his/her independent judgment using a cupping form, where finally the average means of the records of the panelists were used (table8).

Table 4. Physical quality parameters of green coffee beans of Arabica coffee varieties grown in Awi Zone,South Gondar Zone and Zegie Pensiula

Sample code	Flavor	Acidity	Body	TRw+ACV	Total	Final grade
45	7.67	7.5	7.583	79	82.1	Q2
46	7.92	7.92	7.75	76	84.7	Q2
47	8.17	8	7.917	84	85.9	Q1
48	7.83	7.83	7.667	*82*	84.2	Q2
51	7.75	7.75	7.75	79	84	Q2
53	7.75	7.75	7.5	82	83.3	Q2
58	8	8	7.833	82	85.2	Q1
59	7.92	7.83	7.833	79	84.5	Q2
60	7.92	2.92	7.833	82	84.8	Q2
61	8.08	8.17	8.083	82	86.6	Q2
63-B	7.92	7.92	7.75	79	84.7	Q2
64	7.58	7.42	7.33	79	81.7	Q2
65	7.75	7.5	7.667	82	83	Q2
66	7.58	7.67	7.75	76	83.5	Q2
66-A	7.92	7.92	7.833	85	84.8	Q2
66-B	8	8	7.833	82	85.2	Q1
66-E	7.92	7.92	7.917	76	84.9	Q2
66-G	8	8	7.833	83	85.3	Q1
66-H	7.67	7.67	7.5	82	82.8	Q2
67	7.67	7.58	7.667	82	83	Q2
68	7.5	7.42	7.417	79	81.8	Q2
69	7.83	7.83	7.833	85	84.5	Q2
70	7.92	7.75	7.667	82	84.2	Q2
73	7.83	7.75	7.75	85	84.2	Q2
74	7.58	7.58	7.583	79	82.8	Q2
78	7.42	7.33	7.417	76	81.4	Q2
79	7.67	7.67	7.583	76	83.1	Q2

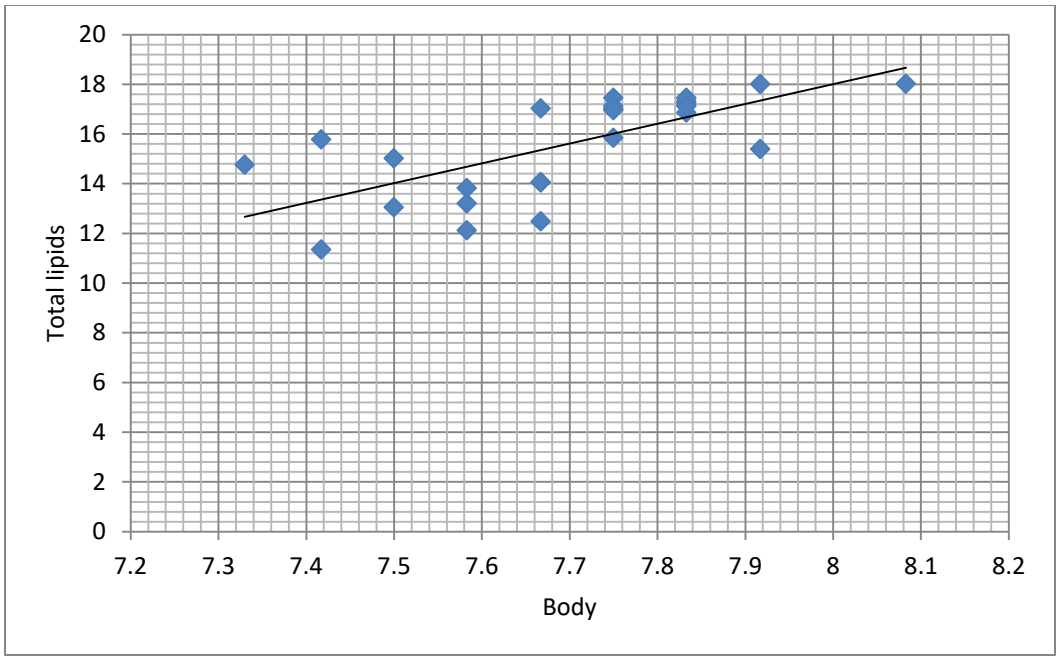
According to the review of (Wale Mengistu et al., 2020), , acidity, body, and flavor were scaled from 0 to 10%. Where 0 = nil, 2 = very light, 4 = light, 6 = medium, 8 = strong, and 10 = very strong. Coffee varieties grown in the highlands of the Amhara Region ranged in acidity, body, flavor, and total coffee quality from 8.16% to 8.66%, 7.66% to 8.33%, 7.66% to 8.16%, and 81.66-87.83%, respectively (Wale Mengistu et al., 2020). The results of this study reported that Arabica coffee varieties grown in three zones ranged in acidity, body, flavor, and total coffee quality from 7.75 to 7.92, 7.66 to 7.83, 7.67 to 7.92, and 82.8 to 84.7%, respectively. The result of this study was argued with reviews by (Wale Mengistu et al., 2020)

Flavor is the most important criterion for coffee quality evaluation and one of the major motivations for consumer preferences(Farah, Monteiro, Calado, Franca, & Trugo, 2006). The coffee flavor, body, total raw and average cup value, and total cup quality had a positive correlation with total lipids grown in the Zegie Pensiula, Awi Zone, and South Gonder Zone had a positive correlation (fig 4). Their correlation coefficients (r) of flavor, body, total raw and average cup value, and total cup quality with total lipids were 0.711786**,** 0.701709, 0.37184, and 0.760151, respectively. This study showed that the total cup quality of green coffee was strongly correlated with total lipids (r = 0.760151) (figure 4-7).



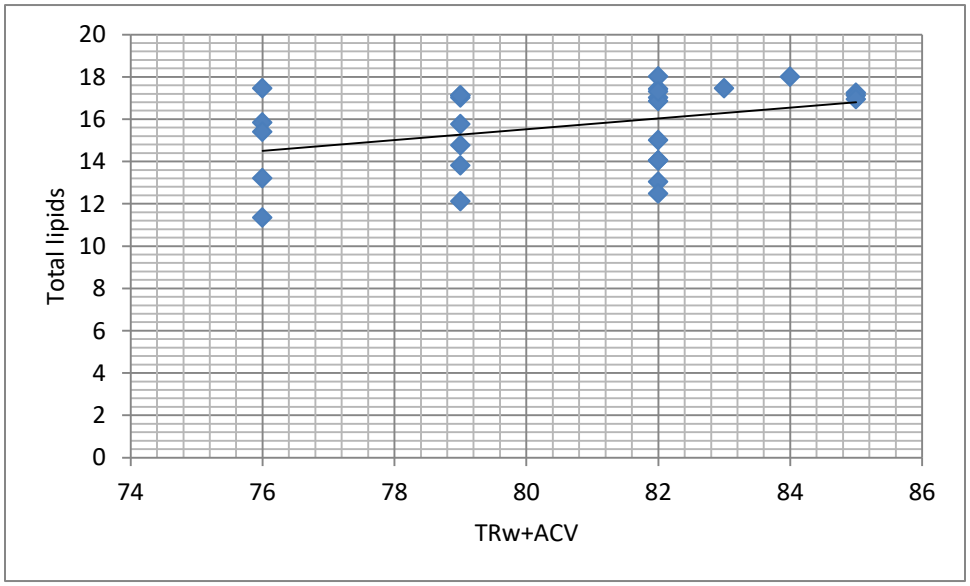
r = 0.711786

Figure 3: Relationship between flavor and the percentage of total lipids extracted from green coffee beans in Awi, South Gondar, and Zegie.



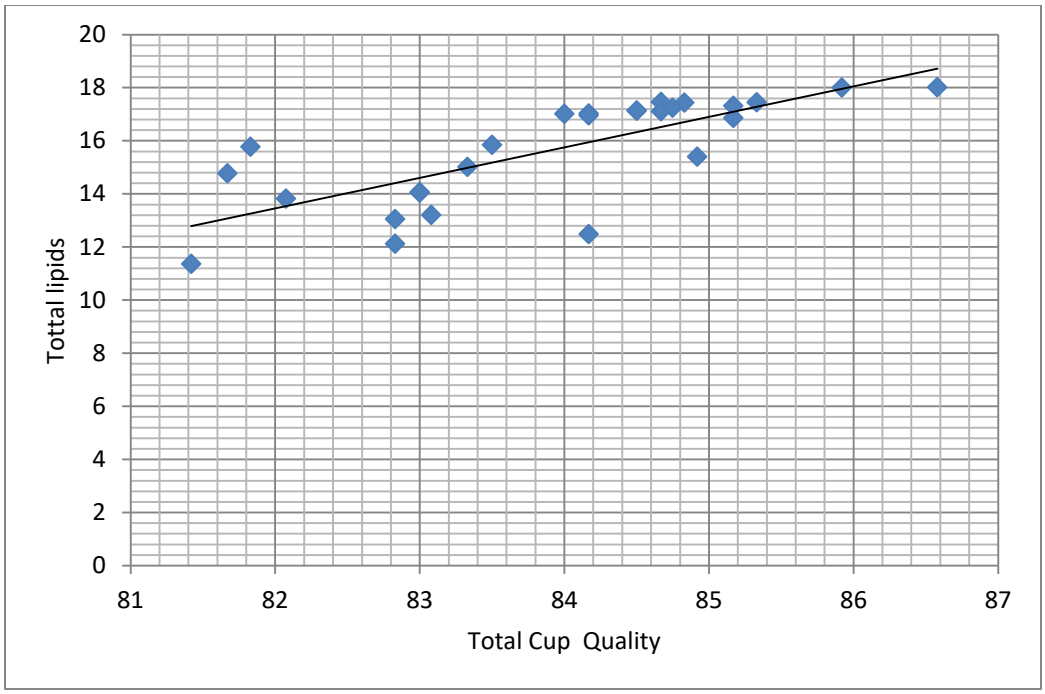
r =0.701709

Figure 4: The correlation between body and total lipids extracted from green coffee beans in Awi, South Gonder Zone, and Zegie



r = 0.37184

Figure 5: shows the relationship between the total lipid extracted from green coffee beans and the total raw value and average cup value.

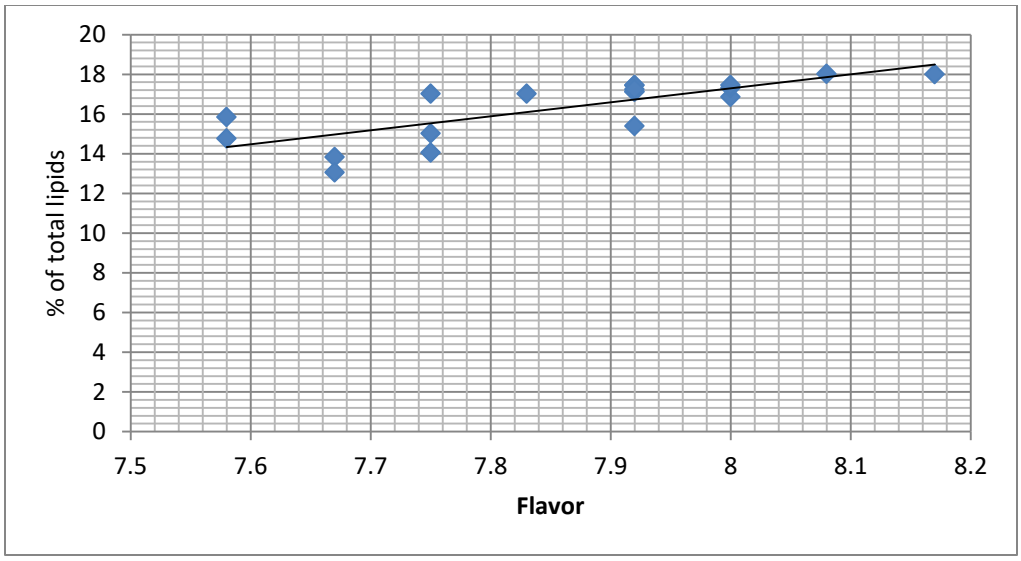


r = 0.760151

Figure 6: The correlation between total lipid extracted from green coffee beans in Awi, South Gonder Zone, and Zegie with total cup quality D

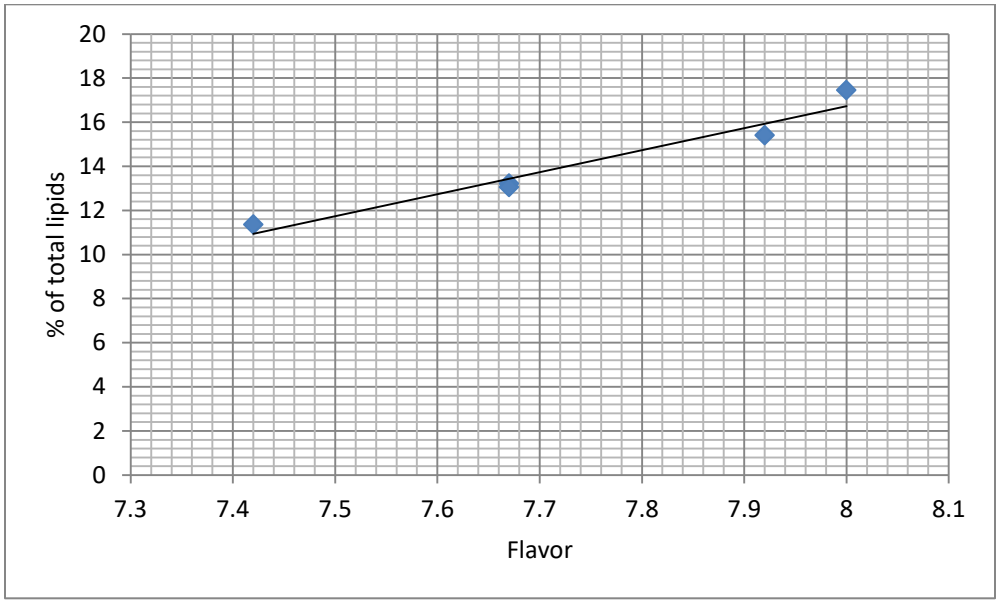
Total lipids in green coffee beans grown in the study areas were positively correlated with flavors (figs. A, B, and C). Figure 8 showed that the Awi Zone had a stronger correlation with coffee flavor than the South Gonder Zone. It had a correlation coefficient of 0.9732. The correlation coefficients (r values) of total lipids with flavors in the Awi Zone and South Gonder Zone of Korata Kebele were 0.9732 and 0.7690, respectively.

The body of green coffee beans and total lipids in green coffee had a positive correlation and were grown in zones. Looking at figure 9, the correlation coefficient values for coffee samples from the Awi and South Gonder Zones are 0.8556 and 0.7333 respectively. This study showed that the body of green coffee beans grown in the Awi Zone had a stronger correlation with total lipids. Figure 10 (E,F), showed that total cup quality, which was grown in the Awi and South Gonder sampling zones, had a positive correlation with total lipids. According to this figure, the correlation coefficient of total cup quality and total lipids in green coffee grown in the Awi Zone,and South Gonder Zone was 0.852 and 0.8437, respectively. This study showed that total cup quality and total lipids had a stronger correlation in green coffee beans grown in the Awi Zone. Their correlation coefficient value was 0.852.



r = 0.7690

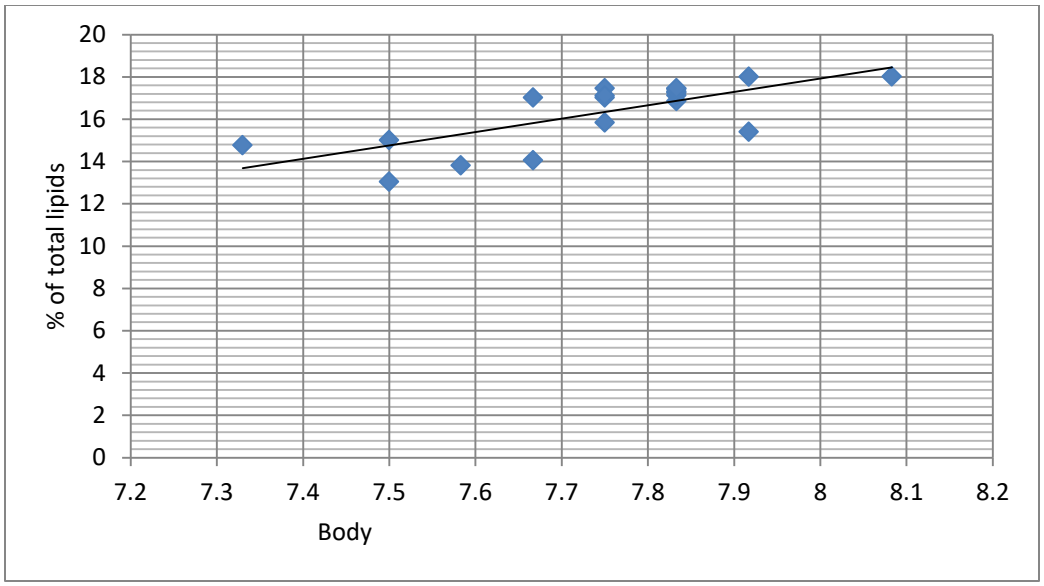
A



r = 0.9732

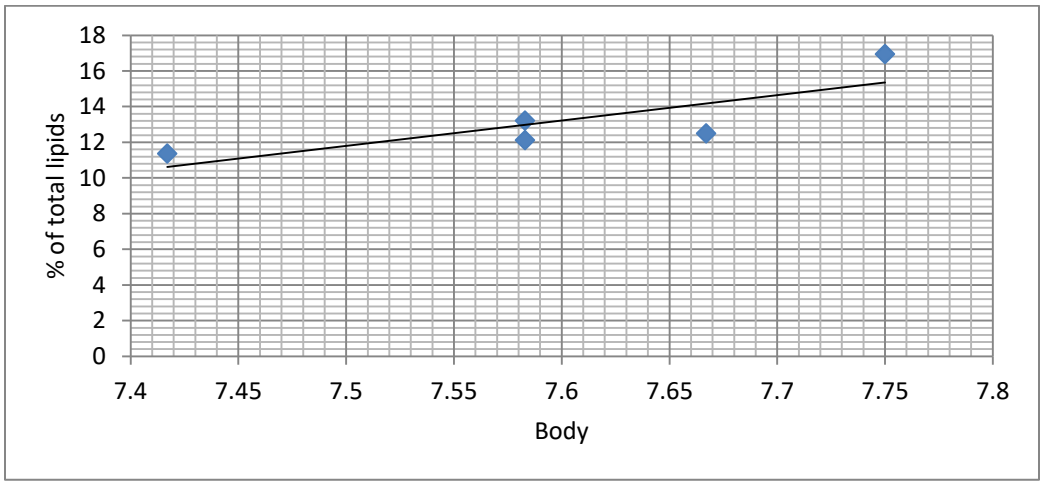
B

Figure 7: shows the relationship between total lipids and flavor in the green coffee beans in South Gonder Zone Korata Kebele Awi Zone (A), and Awi Zone (B).



r = 0.8556

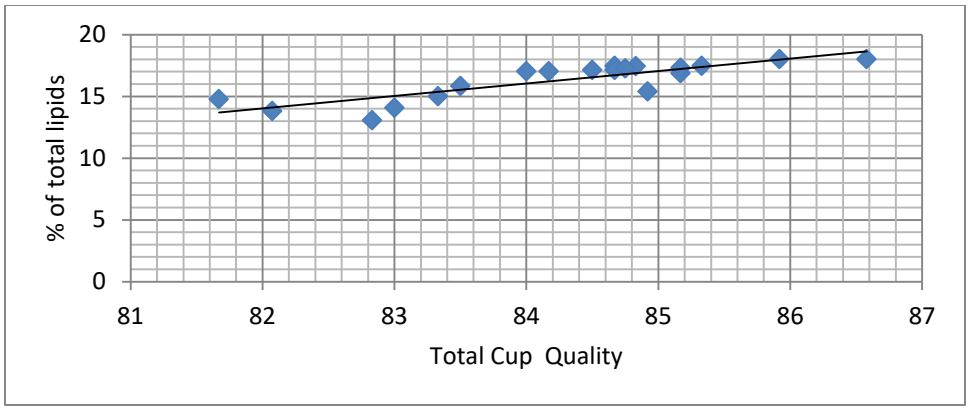
C



r = 0.7333

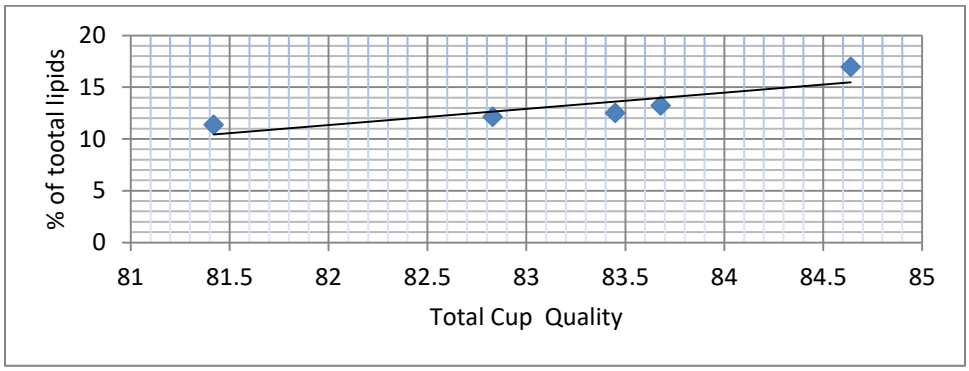
D

Figure 8.Correlation between total lipids with the body of green coffee beans in the Awi Zone (C), and South Gonder Zone (D).



r =0.852

E



r = 0.84370

F

Figure 9: (Eand F). Correlation between total lipids with total cup quality in green coffee beans grown in the Awi Zone (E), and South Gonder Zone Korata Kebele F).

Table8: A summary table of the correlation between coffee quality parameters and total lipids extracted from green coffee beans of three zones is shown.

No	Coffee quality parameter	The correlation b/n coffee parameter with coffee lipids	Correlationcofficent value (r)
1	Flavor	+	0.71
2	Body	+	0.70
3	TRw+ACV	+	0.37
4	Total Cup Quality	+	0.76

4.2. IDENTIFICATION OF THE FATTY ACIDS IN THE COFFEE SAMPLES

A total of twelve fatty acids, eight saturated and four unsaturated fatty acids were detected in all of the green coffee bean samples (Fig10). The identities of the detected fatty acids were determined by comparing the retention times and mass spectral fragmentation patterns by using the NIST spectral library as a reference (table 9).

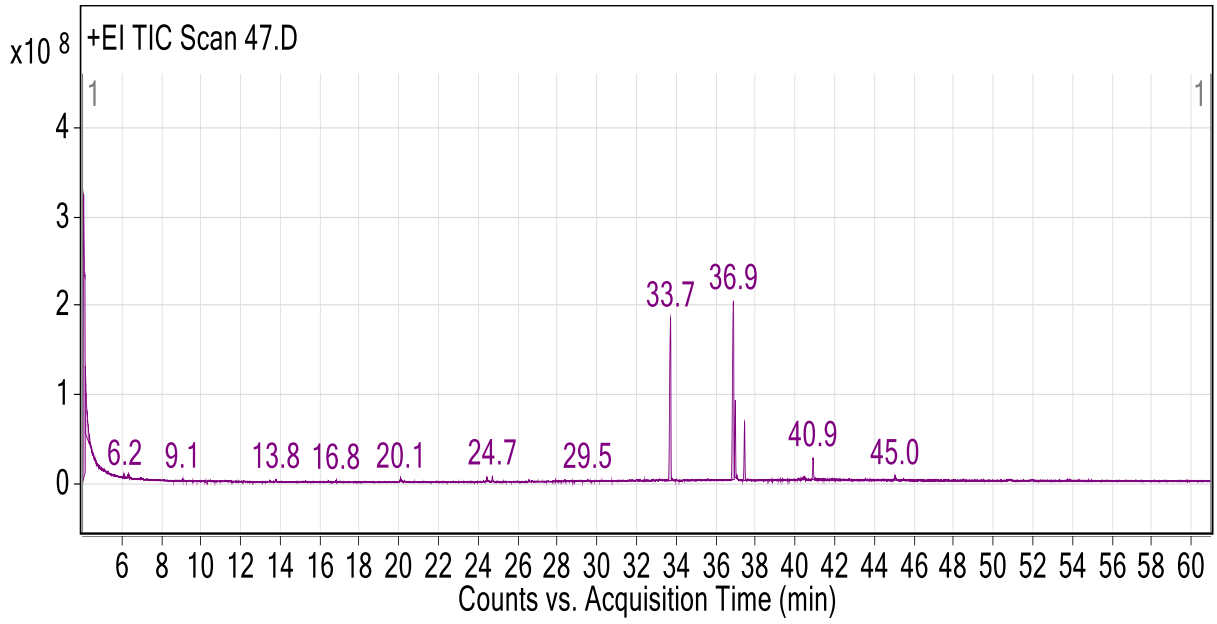


Figure 10: A typical GC-MS chromatogram of Awi Zone green coffee bean extract, indicating the twelve detected fatty acids.

Table9**:** The name,retention time (RT),and meas of identification of fatty acids determined in the green beans

No	Chemical Name	Common Name	Meansof identification	RT	Peak area
1	Pentadecanoic acid	Pentadecylic acid	NIST	31.6	3093048
2	Tetradecanoic acid	Myristic Acid	NIST	31.6	1761342
3	Hexadecenoic acid (Z)		NIST	33.2	4455041
4	Hexadecanoic acid	Palmitic acid	NIST	33.7	906661000
5	Heptadecanoic acid	Margaric acid	NIST	35.6	4545332
6	9,12-Octadecadienoic acid (Z,Z)	Linoleic acid	NIST	36.9	1023552008
7	6-Octadecenoic acid		NIST	37	347875381
8	9-Octadecenoic acid	Oleic Acid	NIST	37.1	5870858
9	cis-11-Eicosenoic acid	Gondoic acid	NIST	40.5	3216676
10	Eicosanoic acid	Arachidic acid	NIST	40.9	99057202
11	Docosanoic acid	Behenic acid	NIST	45.1	28068119
12	15-Tetracosenoic acid(Z)	Lignoceric Acid	NIST	50.9	8566460

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

A statistical analysis using one-way ANOVA was performed to test the presence of significant differences in fatty acid content of coffee beans between the three zones (table 10). According to this table, there was no significant difference in fatty acid content between and within a group of green coffee beans sampled from three zones, and the significance difference was greater than 0.05. Table 10 showed that the contents of fatty acids in green coffee beans sampled from three zones were not varied between and within groups.

Table 10: The significance of fatty acids between and with groups in green coffee from three zones

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Linolic Acid	Between Groups	18.492	2	9.246	1.138	.363
	Within Groups	73.115	9	8.124		
	Total	91.607	11			
Plmitic Acid	Between Groups	10.825	2	5.412	.472	.638
	Within Groups	103.141	9	11.460		
	Total	113.966	11			
Arachidic Acid	Between Groups	.462	2	.231	1.177	.351
	Within Groups	1.765	9	.196		
	Total	2.227	11			
Gondoic Acid	Between Groups	.095	2	.047	2.948	.104
	Within Groups	.145	9	.016		
	Total	.240	11			
Behenic Acid	Between Groups	.162	2	.081	.377	.696
	Within Groups	1.927	9	.214		
	Total	2.089	11			
Margaric Acid	Between Groups	.008	2	.004	.695	.524
	Within Groups	.053	9	.006		Type equat
	Total	.061	11			

4.3. FATTY ACID PROFILE OF GREEN COFFEE BEANS

The fatty acid compositions of green coffee samples from the three study zones are shown in table 11. The amount of linoleic and palmitic acids in Arabica green coffee beans was 44.27–47.31% and 42.87–44.96% of the total fatty acids, respectively**.** According to (Mehari et al., 2019), the amount of linoleic and palmitic acids in Arabica green coffee beans was indicated at 48–57% and 26–33% of the total fatty acids, respectively. Linoleic acid was the most abundant fatty acid in the beans, accounting for 44.27–47.31% of the total fatty acid. In a review by (Mehari et al., 2019), the level of linoleic acid in Arabica green coffee beans was indicated at 48–57% of the total fatty acids. This is in agreement with the present results. The level of

palmitic acid was the second most abundant fatty acid, representing 44.27–47.31% of the total fatty acids.

The most common fatty acids present in green coffee beans in the study of the three zones are shown in the above table: palmitic (C16:0), linoleic (C18:1n-6), arachidic (C20:0), gondoic (C20:1n-9), behenic (C22:0), and margaric acid were detected by GC-MS analysis. Among these, gondoic acid, oleic acid, lignoceric acid, and myristic acid were distinguished in each zone. In addition, gondoic and margaric acid were below 1% of total fatty acids.

The amounts of major fatty acids and linoleic acid in coffee from the Awi Zone, South Gonder Zone, and Bahir Dar special zone (Zegie Peninusla) were 47.31%, 45.91%, and 44.27%, respectively. This acid was higher in samples from the Awi zone (47.31%) than in those from the South Gonder zone (45.91%) and Zegie (44.27%).

The observed variations in the fatty acid content between the green coffee beans from the three different zones can be ascribed to several factors, including genetic trails, harvesting and postharvest processing methods, agricultural practices, and environmental growing conditions (Tsegay et al., 2020). Regarding the postharvest processing conditions, the coffee samples were processed by dry methods in all the sampling areas. However, because of the way the cherries are picked and dried, the samples differed from farmer to farmer. Some farmers used to pick both the ripe and the unripe cherries, while others have selected only the fully ripe (red ripe). Some farmers have followed the right procedures (well-designed beds), while others have used the ground together with other spices. Several investigators have pointed out that genetic properties play a real role in determining the fatty acid content of green Arabica coffee beans (Caporaso et al., 2018).

Furthermore, the amount of linoleic acid content from each individual coffee sample found in the Awi zone ranged from 44.14–50.12%, which is higher than that found in beans from the South Gonder Zone (43.96-49.26%) followed by Zegie (40.26–48.26) . Whereas, the amount of palmitic acid from individual coffee samples is found in the Awi Zone at 40.6-48.23%, which is higher than the South Gonder Zone at 40.6-48%, followed by Zegie at 39.66-46.56%. The variation of linoleic and palmitic acid in each zone of green coffee beans could be attributed to genetic, environmental, or harvesting factors. (Koskei, Mugendi, & Muliro, 2020) Reported that

the content of linoleic acid was one of the most discriminating parameters for coffee variety differentiation between coffee Robusta and coffee Arabica due to genetic variation.

Table 5: the mean, standard deviation, minimum and maximum fatty acid percentages in green coffee from three zones

Zones and numbers of samples										
Fatty Acids	Awie zone = 30			South Gonder = 5			Zegie = 9			
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	P
Linolic Acid	47.3±2.5	44.1	50.1	45.9±2.3	43.9	49.2	44.2±3.5	40.2	48.2	ns
Palmitic Acid	44.9±3.3	40.3	48.2	42.9±3.7	40.0	48.4	42.8±3.0	39.66	46.56	ns
Arachidic Acid	4.6± 0.5	3.9	5.30	4.4 ±0.2	4.1	4.7	4.9± 0.4	4.5	5.5	ns
Gondoic Acid	0.7±0.1	0.5	0.9	0.5±0.1	0.5	0.70	0.52±0.09	0.4	0.6	ns
Behenic Acid	1.3±0.6	0.4	2.0	1.1±0.2	0.8	1.30	1.2± 0.3	0.8	1.5	Ns
Margaric Acid	0.3±0.1	0.2	0.4	0.3±0.1	0.2	0.4	0.23± 0.04	0.19	0.29	ns
Total	98.8±7.3	89.5	106.97	95.2±6.7	89.58	105	94.065±7.357	85.81	102.71	

P = probability level, ns = not significant at $p > 0.05$.

The average fatty acid content (in %) present in green coffee beans in the study of three zones is shown below (Figure 11). GC-MS analysis detected and quantified six fatty acids: palmitic (C16:0), linoleic (C18:2n-6), arachidic (C20:0), gondoic (C20:1n-9), behenic acid (C22:0), and margaric acids. Among these, palmitic acid, linoleic acid, and arachidic acid were distinguished in each zone, i.e., the contents of those average fatty acids in samples from Awi zone were

higher than in samples from South Gonder zone, followed by in samples from Zegie. In addition, margaric acid was found to be a trace amount.

The observed variations in the fatty acid content 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 (Koskei et al., 2020).

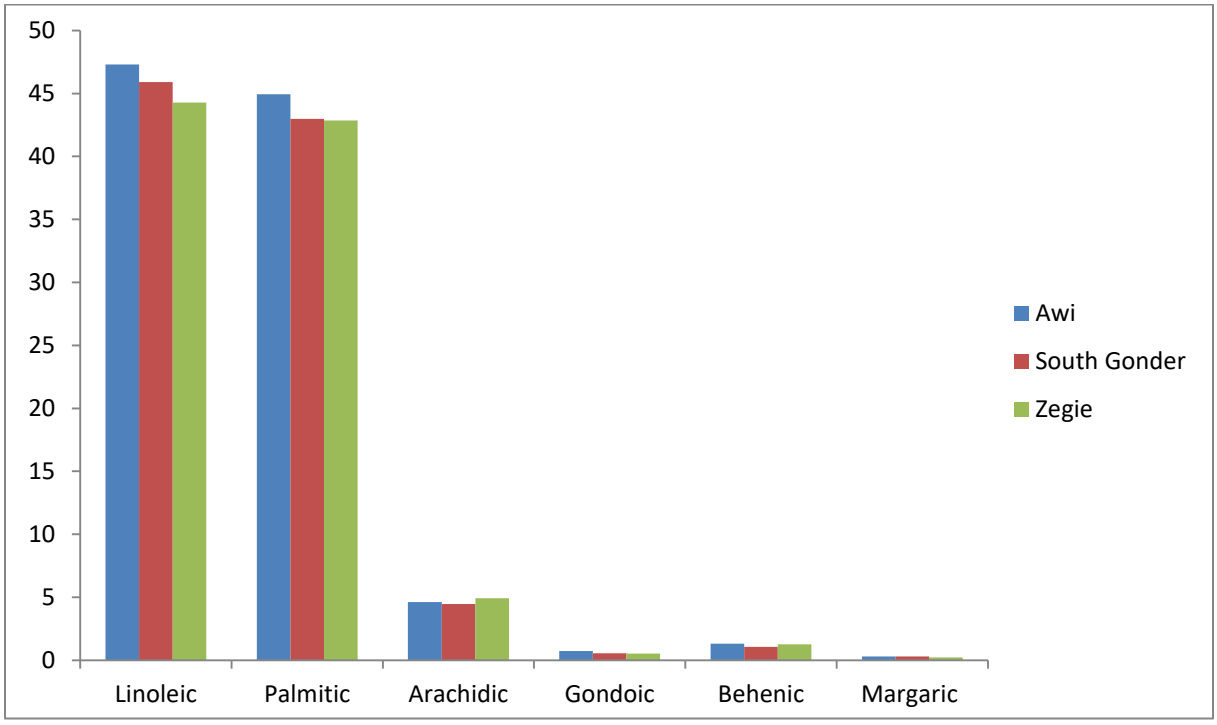


Figure 11: average fatty acid content of green coffee beans in Awi, South Gonder,and Zegie zone samples.

4.4 COMPARISON OF GREEN COFFEE BEANS' FATTY ACID CONSTITUENTS WITH REPORTED VALUES

The highest fatty acid content of green coffee beans in three zones were myristic, pentadecanoic ,palmitic, oleic ,linoleic, arachidic, gondoic, behenic, tricosanoic, and lignoceric acid. Linoleic acid was the most abundant fatty acid in the green coffee beans and accounted for 44.27–47.31% of the total fatty acid content. The level of palmitic acid found in this study ranged from 42.87% to 53.47%. In a review by (Mehari et al., 2019), the level of linoleic and palmitic acids in Arabica green coffee beans was indicated at 48–57% and 26–33% of the total fatty acid,

respectively. Similarly,(Speer & Kölling-Speer, 2006) reported that the green coffee bean from Brazil contained 46–50% linoleic acid . (Koshima, Kitamura, MZ, & Kokawa, 2020), it was reported that the total content of linoleic and palmitic acid in green coffee beans was 53.8- 58.0% and 45.2 - 47.5%, respectively.

The main fatty acids present in all the samples are linoleic acid, with an average percentage of 44.27-47;31%, and palmitic acid, with an average percentage of 42.87-44,96%. Minor acids are myristic, palmitoleic, eicosenoic, and Oleic acids whose contents are lower than 1.0% (Martín, Pablos, González, Valdenebro, & León-Camacho, 2001). The result of this study was in agreement with (Mehari et al., 2019), (Speer & Kölling-Speer, 2006) and (Martín et al., 2001), who reported that linoleic acid was 44.1% and palmitic acid 33.3% in green coffee beans. Other minor fatty acids, such as myristic acid (C14:0), margaric acid (C17:0), gondolic acid (C20:1), oleic acid (C18:1), and lignoceric acid (C24:0), were discovered at percentages of less than 1% (Zhu et al., 2021). The result of this study was in agreement with (Zhu, Long, Ma, et al., 2021).

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 (Koskei et al., 2020).

CHAPTER FIVE

CONCLUSION RECOMMEDATION

5.1 CONCLUSION

In this study, the variation of cup-quality, total lipid, and fatty acids in coffee samples representing selected zones of the Northwestern Amhara region was reported. Among the fatty acids identified in this study, the following were considered significant in all the coffee samples and were considered in a comparative analysis of the coffee samples with respect to the sampling zones: Myristric (C14:0), pentadecylic (C15:0), palmitic (C16:0), oleic (C18:1n-9), linoleic (C18:1n-6), arachidic (C20:0), gondoic (C20:1n-9), behenic (C22:0), Margaric acid, Hexadecenoic acid (Z), 6-Octadecenoic acid ,and lignoceric (C24:0) were the major fatty acids in green coffee beans detected by GC-MS analysis. 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 be comparable with the previously reported literature values. Myristric, palmitic, linoleic, stearic, arachidic, and tricosanoic acids were identified as the most important fatty acids for the 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 coffee flavor, body, total raw and average cup value, and total cup quality had a positive correlation with total lipids grown in the Zegie Pensiula, Awi Zone, and South Gonder Zone had a positive correlation. Their correlation coefficients (r) of flavor, body, total raw and average cup value, and total cup quality with total lipids were 0.711786**,** 0.701709, 0.37184, and 0.760151, respectively. This study showed that the total cup quality of green coffee was strongly correlated with total lipids (r = 0.760151). The total lipids in green coffee beans, which were grown in the Awi zone, were strongly correlated with flavor, body, total raw and average cup value, and cup quality.

5.2 RECOMMEDATION

The fatty acid outline of this study cbe taken as one possible tool for authentication of the region’s coffee.

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