CHARACTERIZATION OF LIPIDS AND EVALUATION OF ELEMENTAL COMPOSITION OF AVOCADO (*Persea americana* Mill) CULTIVARS GROWN IN MURANG'A COUNTY, KENYA

Samuel Njenga Wanjiru

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Chemistry of Murang'a University of Technology

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DECLARATION

Student Declaration

I hereby declare that this thesis is my original work and to the best of my knowledge has not been presented for a degree award of or any award in this or any other University. I understand what plagiarism is and I am aware of the University's Policy in this regard. I understand that any false claim in respect of this work shall result in disciplinary action in accordance with the University Anti-plagiarism Policy.

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Wanjiru Samuel Njenga Date

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Supervisors' Approval

The undersigned certify that they have read and hereby recommended for acceptance of Murang'a University of Technology a thesis entitled **"Characterization of Lipids and Evaluation of Elemental Composition of Avocado (*Persea americana* Mill) Cultivars Grown in Murang'a County, Kenya."**

_____ _____

Prof. Sylvia Awino Opiyo Date

Department of Physical and Biological Sciences

Murang'a University of Technology

_____ _____

Dr. Peter Waithaka Njoroge Date

Department of Physical and Biological Sciences

Murang'a University of Technology

_____ _____

Prof. Beatrice Mugendi Date

Department of Hospitality, Tourism and Travel Management

Murang'a University of Technology

DEDICATION

This work is dedicated to my wife Salome Ngunju, our children James Mwangi, Abigail Wanjiru, Michael Njoroge and MaryAnn Wangari.

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ABSTRACT

Globally, over two billion people suffer from malnutrition and chronic micronutrient deficiencies, representing 7% of the disease burden. Avocado, a nutrient-dense fruit rich in lipids and essential minerals, has potential to alleviate such deficiencies. This study focused on characterizing the lipid content and determining the elemental composition of pulp and seed from four avocado cultivars—Fuerte, Hass, Pinkerton, and Giant—cultivated in Murang'a County, Kenya, a region with optimal climate and soil conditions for avocado farming. Fruits were sampled from three agro-ecological zones (1400–2200 m altitude). Proximate analysis was conducted on fresh pulp, while oil from dried pulp was extracted using petroleum ether. Physicochemical properties of the oil—including specific gravity, acid value, saponification value, iodine value, and peroxide value—were analysed. Fatty acid methyl esters were derived and analysed using GC-MS to determine fatty acid composition. Mineral element profiles in pulp and seed were quantified using Atomic Absorption Spectroscopy. Data were statistically analysed using ANOVA and Tukey's HSD test ($P < 0.05$). Results revealed moisture content ranged from 59.30–84.31%, ash 0.96–1.53%, dietary fibre 1.83–6.43%, and oil 59.44–75.32% among cultivars. Oil properties varied: specific gravity (0.85–0.92), acid value (0.18–0.98 mg KOH/g), saponification value (123.42–187 mg KOH/g), iodine value (54–107 g I_2/100g), and peroxide value (0.9–2.3 meq O_2/kg). The fatty acid profile showed presence of seven fatty acids, predominantly oleic and palmitoleic acids. Fuerte from zone 1 recorded the highest oleic acid content (78.62%), followed closely by Giant (78.52%). Mineral analysis showed potassium (169.2–653.7 mg/100g) and calcium (14.12–49.95 mg/100g) were most abundant in pulp, while iron (0.429–0.786 mg/100g) and zinc (0.049–1.59 mg/100g) were more concentrated in seeds. Statistically significant variations across cultivars and regions led to the rejection of the null hypothesis. The findings demonstrate substantial nutritional variability among avocado cultivars and highlight the potential of Murang'a-grown avocados as sources of health-promoting lipids and minerals. These insights are valuable for nutritional labeling, product development, and informed cultivar selection by producers and consumers.

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ACRONYMS AND ABBREVIATIONS

GDP	-	Gross Domestic Product
UNICEF	-	United Nations International Children's Emergence Fund
WHO	-	World Health Organization
FAO	-	Food and Agriculture Organization
FAOSTAT	-	Food and Agriculture Organization Statistical Data
TAG	-	Triacyl glycerides
FFA	-	Free Fatty Acids
FAME	-	Fatty Acid Methyl Ester
GC-MS	-	Gas Chromatography-Mass Spectrometer
FID	-	Flame Ionization Detector
AAS	-	Atomic Absorption Spectroscopy
AOAC	-	Association of Official Analytical Chemists
ANOVA	-	Analysis of Variance
ASL	-	Above Sea Level
IV-	-	Iodine value
PV	-	Peroxide Value
CODEX	-	International Food Standards, Guidelines and Codes of Practice
FAP	-	Fatty acid profile

CHAPTER ONE

INTRODUCTION

1.1 Background Information

An estimated two billion people globally suffer from malnutrition and chronic deficiency in micro-nutrients and minerals (Gödecke *et al*., 2019). This accounts for 7% global disease burden. In Kenya micro-nutrients, vitamins and minerals deficiency contributes to 53% child mortality and loss of 0.8% GDP annually (UNICEF 2004). One of the strategies employed to reduce micro-nutrients and minerals deficiency is dietary diversification (WHO 2002). This includes consumption of fruits with high levels of phytochemicals, antioxidants, vitamins and minerals.

Avocado (*Persea americana* Mill) fruit is an excellent source of lipids, vitamins, minerals and important phytochemicals (Dreher & Davenport, 2013). Due to its high energy, soft flavor and low sugar content avocado fruit is recommended for patients suffering from diabetes. It also contains high levels of potassium and phosphorous, low levels of sodium hence recommended for hypersensitive patients (Vinha *et al*., 2013).Previous studies have shown that oil and mineral composition of avocado vary with; variety (Indriyani *et al.,* 2016), geographical location of production, maturation (Ferrerya *et al.,* 2016) and part of the fruit analyzed (Ejiofor *et al.,* 2018).

Total lipid content in an avocado fruit may reach up to 30% of the fruit on fresh weight basis (Ge *et al.,* 2017). The profiling of these lipids is important process in determining food quality since each lipid has different nutritional and physicochemical properties (Indriyani *et al*., 2016; Manaf *et al*., 2018). Lipids which contain high unsaturated fatty acids are prone to oxidation leading to undesirable taste as well as compounds that are poisonous to human health (Bhattacharya *et al*., 2008). Oil processors rely on the lipid profile to determine the optimal processing conditions. (Ferreyra *et al.,* 2016).

Avocado pulp contains a lot of minerals despite most studies focusing on lipid profile (Dreher & Davenport, 2013). Avocado seed is about 13-18% of the whole fruit and has high concentration of minerals (Setyawan *et al*., 2021). However, it is discarded during processing since its value is not known/determined leading to accumulation thus environmental pollution. Since goats and sheep eat these seeds, they can be

potential raw materials in animal feeds preparation hence reducing wastage and offer opportunity for income generation.

In Kenya, avocado is mainly grown in the central highlands of Kenya mainly Murang'a, Thika, Embu, Nyeri and Meru regions (Catherine, 2017). Other avocado growing areas are Kisii, Eldoret, Subukia, Nakuru and Naivasha (Shivachi *et al*., 2023). In March 2019, Kenya signed an agreement with the Chinese government to export frozen avocados to china. The China market has the capacity to absorb 40% of the avocado grown in Kenya. Since 2013, Murang'a county government has been supporting farmers with 1,000,000 avocado seedlings every year (Murang'a, 2019). In the last five years 5,000,000 trees have been planted in Murang'a County increasing avocado production and earning Murang'a farmers 2.5 billion shillings annually (Mwambi *et al*., 2016). The Avocado Society of Kenya is however cautioning farmers against producing low quality fruits as this would taint their quality in the export market.

Murang'a County accounts for 53% total avocado produced in Kenya (Catherine, 2017; Muthee *et al*., 2023). The avocados grown in Murang'a County are mainly Hass, Fuerte and local cultivars (Catherine, 2017). Hass represents 70%, Fuerte 25% and local cultivars 5% (Wanjiku. 2021). However, despite avocados being grown in almost all areas of Murang'a County, scanty information on their quality is available.

1.2 Problem Statement

Studies done elsewhere indicated that avocado is an excellent source of vitamins and micro-nutrients, and is a "functional food". Murang'a County has diverse climate and grows an abundance of native avocado cultivars. However, there is little published data on the quality of these various avocado cultivars cultivated in the County. Rural poverty and food and nutrition insecurity of small-scale farmers necessitates the need to improve agricultural production capacity, economic potential and nutritional outcomes. Avocado seed is also under-utilized which makes it necessary to determine its economic potential further reducing wastage. Malnutrition due to lack of essential fatty acids and micro-nutrients necessitates the need to investigate the avocado fruit as a potential source of these nutrients.

1.3 Objectives of the study

1.3.1 General Objective

To characterize lipids and elemental composition of different avocado cultivars grown in Murang'a County

1.3.2 Specific Objectives

i. To determine the percentage moisture, ash, dietary fibre and oil content of different avocado cultivars grown in Murang'a County.
ii. To determine the physicochemical properties of the oil extracted from the pulp of different avocado cultivars grown in Murang'a County.
iii. Evaluate the fatty acid composition of oil from different avocado cultivars grown in Murang'a County.
iv. Evaluate elemental (K, Na, Ca, Mg, Zn, Fe, Cu) composition of the pulp and seed of different cultivars of avocado grown in Murang'a County.

1.4 Hypotheses of the study

i. H_{01}.There is no significant difference in lipid, moisture ash and dietary fiber content of different avocado cultivars grown in Murang'a County.
ii. H_{02}. There is no significant difference in the physicochemical properties of oils extracted from different avocado cultivars grown in Murang'a County.
iii. H_{03} There is no significant difference in fatty acids composition of Avocado cultivars grown in Murang'a County.
iv. H_{04} There is no significant difference in elemental composition of the pulp and the seed of different avocado cultivars grown in Murang'a County.

1.5 Contributions of the Thesis

The main contributions of the study are: The study provides new and specific data on the chemical composition, lipid content, and elemental composition of different avocado cultivars grown in Murang'a County, where such information was previously scarce. The research produces a new body of empirical data on the proximate composition, physicochemical properties, and elemental profiles of these avocados. The study's findings shed new light on how these avocado cultivars can be used for

nutraceutical, cosmetic, and industrial purposes, which is a new piece of information for the field.

1.6 Significance of the Study

Physicochemical properties of oil are important parameters in determining the quality of oil. They provide baseline information that is used in determining the commercial application of oil as well as designing processing technologies for applications of avocado oil in industries like cosmetics, pharmaceuticals, vegetable oil and other individual uses.

Due to increased avocado consumption, there is increased seed waste generation therefore waste management is essential. Analysis of mineral elements in the seed is important in determining its possible applications such as supplementation in animal feeds. This would improve the economic value of the avocado and also reduce pollution.

1.7 Scope and Limitation

The study was carried out in Murang'a county Kenya. Fresh avocado fruits were picked from three different agro-ecological zones in Murang'a County. These are; the eastern region with an equatorial type of climate, the central region with a sub-tropical climate and the eastern part with semi-arid conditions. Four common cultivars of avocados grown in Murang'a County were analysed. These are Fuerte, Giant, Hass, and Pinkerton. The study focused on determining the physicochemical properties and fatty acid profile of oil extracted from the pulp of ripe avocado fruits. The study analysed the following significant physicochemical properties: specific gravity, refractive index, acid value, saponification value, iodine value and peroxide value. The following selected mineral elements in both pulp and seed were determined: K, Na, Ca, Mg, Zn, Fe, and Cu.

1.8 Organization of the Thesis

The content of this research thesis is organized into five chapters as highlighted below: Chapter one is introduction. This chapter provides a foundational overview of the research study, beginning with a detailed background and a review of existing literature. This leads to a clear problem statement, which highlights the gaps this study aims to address. The research objectives are then articulated, including a general

objective and several specific ones, which are directly tied to the testable null hypotheses. The chapter concludes by outlining the expected contribution of the thesis to the field and discussing the significance of the study, explaining its potential impact.

Chapter two is literature review. This chapter reviews existing literature on avocados, covering global and Kenyan production trends, with a specific focus on Murang'a County. It discusses various avocado cultivars, the fruit's nutritional value, and methods for determining its quality based on moisture, oil, ash, and fiber content. The chapter also examines the physicochemical characteristics and fatty acid composition of avocado oil, as well as the elemental makeup of both the pulp and seed. It concludes by detailing advanced analytical techniques like GC-MS and AAS used for analysing avocado oil and elemental composition. The purpose of this review is to provide a foundation for the study and identify areas for future research.

Chapter three is materials and methods. This chapter presents a comprehensive description of the materials, methods, and experimental procedures used in this research. Its primary goal is to provide a clear and reproducible account of the scientific approach, enabling others to validate the findings and replicate the experiments. The methodology systematically covers all aspects of the study, beginning with the detailed description of the study site and research design. It then outlines the procedures for sampling, sample preparation, and the use of specific reagents. Subsequent sections detail the analytical methods for proximate analysis, lipids extraction, and the determination of various physicochemical properties of the oil. The chapter concludes by describing the advanced techniques for determining fatty acid and elemental compositions, as well as the statistical methods used to analyse the data.

Chapter four is results and discussion. This chapter systematically presents and interprets the findings from the experimental procedures detailed in Chapter Three. The results are organized to address the study's objectives, beginning with the proximate composition of the samples, which includes moisture, ash, and lipid content. This is followed by a detailed discussion of the physicochemical properties of the extracted oils, such as specific gravity, acid value, iodine value, saponification value, and peroxide value. The chapter concludes with the presentation and analysis of the elemental composition, focusing on key minerals like potassium, sodium,

calcium, magnesium, iron, zinc, and copper. Each section provides a comprehensive discussion of the data, relating the findings to existing literature and highlighting their significance

Chapter five is conclusions and recommendations. The chapter is structured to first present a comprehensive conclusion that encapsulates the significant findings regarding the avocado cultivars from Murang'a County. This is followed by a section dedicated to recommendations, which are categorized into three distinct areas. The first set of recommendations focuses on nutrition applications, suggesting how the findings can be practically applied to improve dietary practices. The second set addresses policy recommendations, proposing actions for relevant governmental and non-governmental bodies. Finally, the third set provides recommendations for future research, identifying potential avenues for further investigation to build upon the foundation laid by this thesis highlighting their significance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter reviews existing literature on avocados, covering global and Kenyan production trends, with a specific focus on Murang'a County. It discusses various avocado cultivars, the fruit's nutritional value, and methods for determining its quality based on moisture, oil, ash, and fiber content. The chapter also examines the physicochemical characteristics and fatty acid composition of avocado oil, as well as the elemental makeup of both the pulp and seed. It concludes by detailing advanced analytical techniques like GC-MS and AAS used for analyzing avocado oil and elemental composition. The purpose of this review is to provide a foundation for the study and identify areas for future research.

2.2 Avocado production

Avocado scientifically known as *Persea americana* Mill is a tropical ever green climacteric fruit that belongs to the family of *Lauraceae* of tropical and Mediterranean trees and shrubs, and it is the only species that produce edible fruits (Ikhuoria *et al.,* 2007). Avocado originated from Mexico, South and Central America (Dreher & Davenport, 2013). There are three known ecological avocado races namely Mexican race (*Persea americana* var. drymifolia), Guatemalan race (*Persea americana* var. guatemalensis) and West Indian race (*Persea americana* var. Americana) (Manaf *et al.,* 2018).

Avocado, is a widely consumed fruit that is rich in minerals and fats (Colombo & Papetti, 2019). Avocado farming has increasingly been adopted in recent years in over 57 countries, especially small-scale farmers who account to over 70 percent of the total production. According to reports published by FAOSTAT (2020), over the past decade, avocado production has doubled from 4.07 million tonnes in 2011 to about 8.06 million tonnes in 2020. This represents approximately 50.46% increase in avocado production since 2011. Figure 2.1 shows the production of avocado globally from 2011 to 2020 (FAOSTAT 2018). The high uptake of avocado by farmers has been linked to the huge profitability especially from the international markets together with the global awareness of its health benefits.

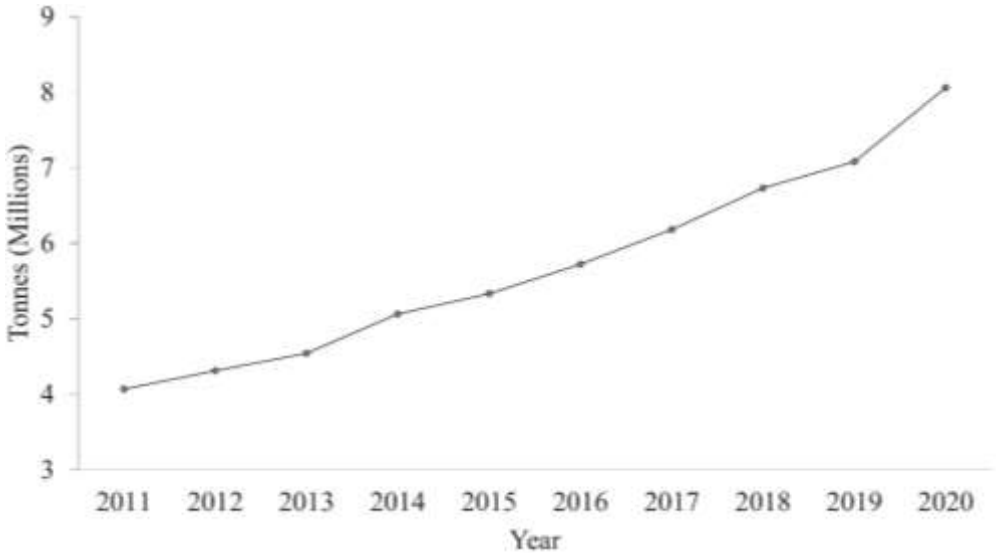


Figure 2.1: The production of avocado globally between 2011 and 2020 (FAOSTAT, 2020)

Based on production per country, Mexico is the highest producer and exporter of avocado fruits and has remained so at least for the past decade, followed by Colombia and Peru as the second and third producers with a production capacity of 2,393,849, 829,147 and 672,232 tonnes, respectively (FAOSTAT, 2020) (Table 2.1). The world's top eleven avocado-producing countries are Mexico, Colombia, Peru, Dominican Republic, Indonesia, Kenya, Brazil, Ethiopia, United States of America, Haiti, and Chile (FAOSTAT, 2020) (Table 2.1). The leading producers of avocado fruits in Africa include Kenya, Ethiopia, Malawi, South Africa, Cameroon and Morocco, with an annual production of 322,556, 245,336, 93,565, 84,775, 74,871, and 69,940 tonnes respectively (FAOSTAT, 2020).

Table 2.1: Avocado production among the top producing countries in 2020 (FAOSTAT, 2020)

No.	Country	Production (Tonnes)
1	Mexico	2393,849
2	Colombia	829,147
3	Peru	672,232
4	Dominican Republic	620,087
5	Indonesia	609,049
6	Kenya	322,556
7	Brazil	266,784
8	Ethiopia	245,336
9	United States of America	187,433
10	Haiti	179,333
11	Chile	161,210

According to FAOSTAT (2020), Kenya produced 322,556 tonnes of avocado in 2020 (Table 2.1). Over the past decade, avocado production in Kenya has been increasing steadily from 149,241 tonnes in 2011 to 322,556 tonnes in 2020 (Figure. 2.2). This represents about a 216.13% increase in avocado production since 2011.

2.3 Avocado Production in Kenya

Avocado is primarily grown for the fresh fruit market, either domestic or export. There are also industries for process in avocados such as avocado oil mainly for different applications: food market and cosmetic industry. Due to its nutritional value many Kenyan farmers are growing the crop more and more to meet the local and export market demand. The expansion of avocado growing has made Kenya the 6th world largest exporter of the fruit (Wasilwa *et al*., 2017). Figure 2 shows the production of avocado in Kenya from 2011 to 2020. Kenya produces on average 115 000 metric tonnes of avocados and has earned 5.2 billion in 2015 and 6.2 billion in 2016 (USAID, 2017; Ministry of Agriculture (MoA), 2017).

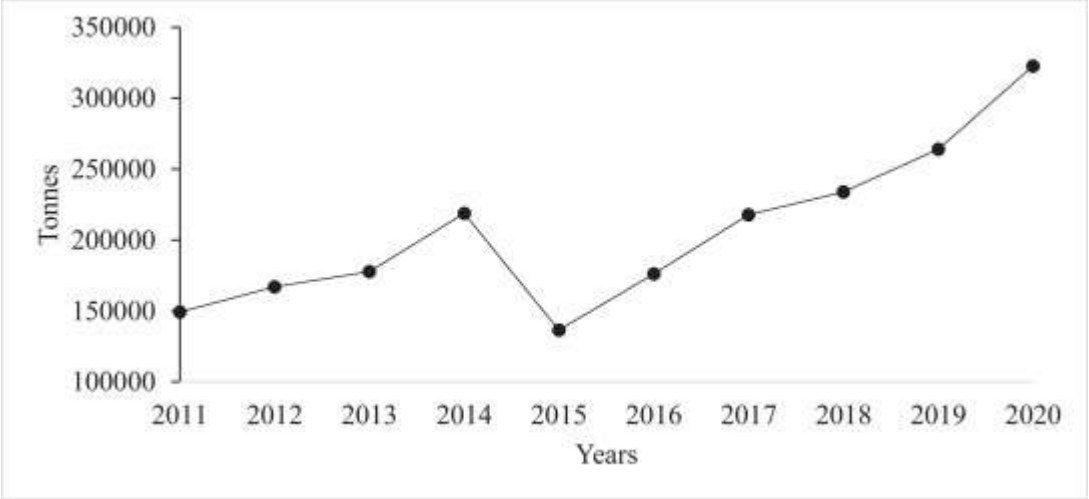


Figure 2.2: Production of avocado in Kenya since 2011 (FAOSTAT, 2020)

2.4 Avocado Cultivars Grown in Murang'a County

Nowadays, avocados is found distributed throughout the world where it is grown in tropical and sub-tropical areas (Ahmed *et al.,* 1980, Nasri *et al.,* 2023). Currently there are 12 major cultivars of avocado which are hybrids of the three races; Hass which is Guatemalan-Mexican hybrid is the main export variety while Fuerte is preferred for oil processing (Chanderbali *et al.,* 2008). Other commercial cultivars are Keitt, Reed, Booth 8, Simmonds, Pinkerton, Nabal, Puebla, Tonnage, Ettinger, Hayes, G6 and G7. Cultivars used as rootstocks include Puebla, Fuerte, Duke, G6, and G7.

The selection of any avocado cultivar should consider the quality of the desired fruit and the tree characteristics (Nasri *et al.,* 2023). Every avocado tree grown from a seed can be used as a start of a cultivar by grafting to suit the climate and geographical growing area. This information is important to farmers since they are able to convert from one cultivar to another depending on the needs and climate variability.

There are five main avocado cultivars grown in Murang'a County (Catherine, 2017). Hass and Fuerte are the main cultivars for commercial purposes. Golden, Giant and Pinkerton are the other cultivars grown in low quantities. Hass is the main variety that Murang'a county government is supporting farmers by providing them with seedlings. Internationally, Hass has better acceptance due to its excellent properties and it has higher resistance to post harvest processes that enable the fruit to reach the consumer at a higher price. Murang'a County has varied climatic conditions and soil types hence, the need to studies the suitability of growing certain cultivars in specific areas

(Charles, 1999). Avocados produced in Murang'a County are as different as the geographical area in which they grow (Muturi, 1990). The suitability of any avocado variety should be based on variety characteristics as observed in one or more locations and does not guarantee that the variety is adapted to all locations (Yanty *et al.*, 2011).

Fuerte

This variety was discovered in 1911 as a garden seedling in Atlixo, Mexico. It is believed to be a cross between a Mexican and a Guatemalan kind, yielding group B flowers. Fuerte is currently the most well-known commercial cultivar in the world and possesses all the attributes that both domestic and international markets seek. The average-sized green pear-shaped fruit weighs between 290 and 380 g, and its relatively small seed is firmly enclosed in its cavity. The flesh, which is pale yellow and nearly devoid of fibre, has a great flavour. The skin is thin, leathery, and slightly rough. The seed easily separates from the meat and has an oil content of 16–25%. There may be multiple sets of fruits on the trees at the same time because Fuerte blooms for a long time. Because of its spreading growth habit, which sets it apart from most other cultivars, Fuerte should be spaced at least 8 m by 10 m (Christopher *et al*., 2014; Biazin *et al*., 2018).

Hass

This variety was discovered in 1920 where a random seedling was chosen to become the original tree at La Glabra Heights, California. Due to its exceptional ability to adapt to a variety of environmental conditions, this Guatemalan cultivar has gained prominence in newly planted areas worldwide. Its picking season is later than Fuerte's and its bloom type is A. The mature fruits have creamy yellow flesh that is devoid of all fibre and are of exceptional quality. When the fruits are almost mature, they turn from green to a dark blue colour. They have a hard, pebbly skin and are ovoid to pear-shaped, weighing up to 340 g. Since the early 1970s, Hass fruits have been widely accepted by consumers, ship well, and have a longer shelf life. The round seed is small and separates readily from the flesh which has a fat content of 18 – 23% resulting in an excellent nutty flavour. The variety is a vigorous, moderately spreading tree. It comes into cropping at an early age and usually bears regularly and heavily. Recommended spacing is about 7 m x 8 m (Christopher *et al*., 2014; Biazin *et al*., 2018).

Pinkerton

This is a relatively new cultivar that was developed in 1970 as a Rincon seedling on the Pinkerton Ranch in Ventura County, California. It is believed to be a Mexican and Guatemalan hybrid that produces type A flowers. Usually weighing between 250 and 410 g in the mid- to late-season, this tall, pear-shaped cultivar has a dark green color. The meat is smooth, fine, and creamy, with a high oil content (18–20%), and the outside is medium thick, leathery, and pebbled. The tiny seed readily separates from the flesh, but the covering adheres to it. The fruit does not get damaged easily during transportation and has a good shelf life. Strong, regular, and early bearing characterize this medium-sized tree, which spreads more than Hass. Six meters by six meters should be the recommended planting distance (Christopher *et al*., 2014; Biazin *et al*., 2018).

Giant

This accidental seedling was discovered in 1970 in Kenya's Trans Nzoia District on the Wanguhu farm. It is said to be a hybrid from Guatemala that yields type A flowers. The medium-sized, pear-shaped, dark-blue/black fruits ripen late and are of very high quality. Fruits often have a thick, pebbly skin and weigh between 210 and 355 g. The comparatively small seed easily separates from the mature fruit together with its seed coverings. The fruit contains 18–25% oil content. The tree is big and leaning. It bears fruit at a young age, and its crops are consistent and heavy to excellent. An approximate spacing of 8 m by 8 m is advised (Christopher *et al*., 2014; Biazin *et al*., 2018).

2.5 Nutritional Value of Avocado

An estimated two billion people globally suffer from malnutrition and chronic deficiency in micro-nutrients and minerals (Fanzo *et al*., 2018; Fongar *et al*., 2019) accounting for 7% global disease burden (Muthayya *et al*., 2013; Fanzo *et al*., 2018; Gödecke *et al*., 2018; Lenaerts & Demont, 2021). In Kenya, micro-nutrients, vitamins and minerals deficiency contributes to 53% child mortality and loss of 0.8% GDP annually (Fongar *et al*., 2019). One of the strategies employed to reduce micro-nutrient and mineral deficiency is dietary diversification (Organization, 2003) including taking fruits with high levels of phytochemicals, antioxidants, vitamins and minerals (Bisht *et al*., 2018; Comerford *et al*., 2021; Ihesinachi & Eresiya, 2014; Li *et al*., 2021).

Plants have for long provided humanity with their basic necessities, including food, medicine and shelter (Jeruto *et al*., 2017). Plants are the primary source of variety of vitamins, minerals, and other essential nutrients that enter the food chain. In addition, plants contain phytochemicals and antioxidants that help keep the body in balance and the immune system functioning properly and are harmful to infectious diseases (Opiyo *et al*., 2021). In recent years, there has been an increase in the search for natural alternatives to synthetic chemicals used in the food, pharmaceutical, pesticides, and cosmetic industries (Njoroge *et al*., 2019). Plant materials and extracts are recommended for food and infections control since they are safe for non-targeted organisms and the environment (Opiyo *et al*., 2023). Furthermore, the risk that hazardous microorganisms may develop resistance to herbal treatments is exceedingly low.

Avocado is a fruit with a characteristic good taste and aroma due to the presence of unsaturated fatty acids (Arukwe *et al*., 2012; Arackal & Parameshwari, 2017). Avocado pulp contains quality lipids such as omega-3, omega-6 and omega-9 fatty acids which are inversely related to heart diseases (Carranza *et al*., 1997). Studies have shown that avocado oil has a high digestibility coefficient greater than 93.8%. It is therefore used as salad oil. Avocado is a low sugar fruit with high proteins, fibre and vitamins A, B, C and D. Lipids found in the avocado are sources of energy and can replace carbohydrates thus helping reduce triglycerides found in blood plasma in patients with diabetes. Avocado pulp also contains antioxidants (vitamin E/tocopherol) and glutathione that neutralize free radicals that may damage aging cells and the heart. Carotenoids in the pulp protects the eyes from diseases such as eye cataracts.

Minerals found in avocado such as copper, zinc and iron are essential elements necessary for maintaining good health (Dreher & Davenport, 2013; Vinha et al., 2013). Calcium, phosphorous and magnesium helps in maintaining the body's water balance, while potassium and sodium helps in the metabolism of fats and maintain better pH of the cells. Low levels of sodium make the avocado be recommended to patients who are hyper-sensitive (Nair & AC, 2018). Avocado is a "functional food'' and its inclusion in everyday diet delivers other non-nutritive advantages that may enhance good health (Mazza 1998; Arackal & Parameshwari, 2017; Weschenfelder *et al*., 2015).

2.6 Avocado Quality Determination

The world market is also seeking healthy and quality products. In this light a number of food quality assessment organizations locally and internationally have been established to certify and confirm the composition of food and agricultural products. This can only be successful if the chemical characteristics of the products are well known. In recent past a number of food quality systems with regard to agricultural foodstuffs and products have been established (Carvalho *et al*., 2015; Teng *et al*., 2016). Avocado Society of Kenya and KEPHIS are actively monitoring the quality of avocado fruits to fully comply with global standards and regulations by educating farmers and marketing the products for them. In this line Murang'a avocado farmers have been cautioned against selling immature fruits as it would taint the quality of Kenyan avocado in the European market.

Several researchers have studied different components of avocado pulp in order to determine their quality. Some of the components studied are; maturity index (Ozdemir and Topuz, 2004; Donetti and Terry, 2014), oil content (Ozdemir and Topuz, 2004; Takenaga *et al*., 2008; Ariza *et al*., 2011; Donetti and Terry, 2014), digestibility coefficient and amount of minerals in the pulp (Maitera *et al*., 2014). These components have been found to vary from season to season for each cultivar, geographical growing area, maturity, soil conditions and farm management practices. This study investigated the variation of proximate composition of the pulp, physicochemical properties of oil extracted from the pulp, fatty acid composition and elemental composition of four different cultivars grown in three different geographical areas of Murang'a County.

2.6.1 Moisture Content

Avocado is characterized by great natural variability. The question of when to start harvesting avocado fruit is of great commercial importance. Avocado fruits should be allowed to maintain sufficient maturity to be palatable upon ripening (Yanty *et al*., 2011). It is difficult to determine the harvesting period especially for green- skinned cultivars (Donetti & Terry, 2014). Farmers should therefore know when to pick and how to pick the fruits. Maturity of avocado in several countries is determined by chemical analysis.

According to Lu *et al.* (2008), the most widely used indicator for avocado fruit maturity is the measurement of moisture or dry matter content. Nonetheless, other complementary indicators may be taken into account, such as the softening of the flesh and the turning of the green skin to black in certain cultivars, like "Hass" (Maitera *et al*., 2014). Dry matter is dependent on variety, region and season (Teng *et al*., 2016). This approach to assessing avocado maturity is more precise. In Kenya, the minimum requirements for the maturity of fresh fruits and the processing of oil are 24% and 30% dry matter, respectively. As the fruits mature, there is more dry matter.

2.6.2 Oil Content

Percentage oil content determination has also been another common indicator used for avocado fruit maturity (Hofman *et al*., 2002; Galvao *et al*., 2014). The amount of oil depends on cultivar, maturity, geographical growing area, ripening stage and extraction method. The California Avocado Industry in the United States started using a minimum standard of 8% oil content in the pulp of avocado fruits in 1925 (Gomez-Lopez, 1998). However, starting in the 1980s, they started using minimum oil content percentages for each cultivar: 10.0% for "Fuerte" and 11.2% for "Hass" (Lee *et al*., 1983; Dodd *et al*., 2010).

In a study of oil content of three cultivars namely *Hass, Breda* and *Margarida*, there was significant difference in the percentage oil content among cultivars and different ripening stages (Carvalho *et al*., 2015). In another study on fatty acid composition of oils from four avocado cultivars namely *Quintal*, *Fortuna*, *Margarida* and *Hass* similar observations were made (Zaenab Alnasan *et al*., 2019). Avocado harvested in early, mid and late season yielded significantly different percentage of oil (Teng *et al*., 2016).

In solvent extraction the pulp is first dried and the dried pulp combined with a suitable solvent in an extractor and then the solvent separated from the oil. When choosing a solvent, caution is necessary because some are flammable and volatile, while others give oil an unpleasant smell that needs to be eliminated. Researchers consequently select solvents that yield the most precise oil content in the dry pulp. According to a study by Muturi (1994), on the most effective solvents in determining the percentage oil content from fruits of average maturity, the following results were obtained; Water 2.67-3.12%, acetone 0%, methanol 0%, n-hexane 8.24%, cyclohexane 11.21%,

petroleum ether (PE) 13.55 %, ethanol 12.14 %, and carbon tetrachloride 6.20%,. Petroleum Ether gave the highest percentage oil content and was considered the most effective.

2.6.3 Ash Content

Avocado fruit is rich in minerals (Dreher & Davenport, 2013; Vinha *et al*., 2013). Studies carried out in four commercial cultivars in Mexico (the highest producer of avocado in the world reported that the amount of ash content determines the amount of nutrients and not the size of the fruit or the amount of harvest (Hofman *et al.*, 2002). The report concluded that growers should expect significant difference in nutrients in different avocado cultivars. Growers should therefore determine the yield and the quality, by determining the nutrients composition in any avocado fruit among the different cultivars. Similar observations were made on heavy metal analysis on selected fruits in Ethiopia where fruits shown different concentration of the elements depending on the area of origin (Tegegne *et al.*, 2017).

2.6.4 Dietary Fibre

Dietary fibre is found in wholegrain cereals and fruit and vegetables. Fibre is made up of the indigestible parts or compounds of plants, which pass relatively unchanged through our stomach and intestines. Fibre is mainly a carbohydrate whose primary function is to maintain the digestive system's health. Constipation, hiatus hernia, appendicitis, diabetes, obesity, coronary heart disease, gallstones, and other conditions are mainly brought on by diets lacking in dietary fibre (Sudha *et al*., 2011). The risk of the diseases listed above is potentially decreased when sufficient levels of dietary fibre are consumed (Academy of Nutrition and Dietetics, 2015). Studies have shown that avocado oil has a high digestibility coefficient greater than 93.8 %. It is therefore used as salad oil (Carranza *et al*., 1997). Studies have shown that dietary fibre vary by the cultivar, the part of the fruit analyzed (Galvao *et al*., 2014) and geographical growing area (Maitera *et al*., 2014).

2.7 Physicochemical characteristic of the oil

Physicochemical properties of oil are an important variable in considering its applications in life as they influence its quality. Physicochemical properties like acid value, iodine value, saponification value, peroxide value provide information in determining the suitability of oil for consumption (Bamgboye *et al.*, 2010; Parthibanet

al., 2011). Further when designing technological processes physicochemical properties are important parameters to manufacturers (Bhattacharya *et al*., 2008).

Oils contain various triglycerides in different proportions. Specific gravity and viscosity depend on the type of triglycerides present. Specific gravity and viscosity decrease with increase in saturation and increases with unsaturation and polymerization. Refractive index of an oil is the ratio of speed of light at a defined wavelength to its speed in the oil/fat itself. This value varies with wavelength and temperature, the degree and type of unsaturation, the type of substitutions of component fatty acids and with accompanying substances (Nichols and Sanderson, 2003). Refractive index is widely used in quality control to check for the purity of materials and to follow hydrogenation and isomerization of an oil (Khodier, 2002).

Acid value of an oil indictaes the amount of free fatty acid present that affect its stability and quality. It is measured by milligrams of KOH required to neutralize the fatty acids in one gram of lipid. Acid value is one metric used to assess an oil's edibility and suitability for industrial uses like paints is its acid value (Akubugwo *et al*., 2008). An oil with a low acid value is stable for a long time and is protected from peroxidation and rancidity. The avocado pulp's natural antioxidants, including vitamins A and C, as well as other potential phytochemicals, like flavonoids, may be the cause of this (Gordon *et al*., 1993). Although an oil with a high acid value may not be edible for use in cooking, it can be helpful for making paints, liquid soap, and shampoos (Akintayo, 1997; Aremu *et al*., 2006). Additionally, oils with a noticeable acidity level are a sign that the plant may be toxic to cattle (Aremu *et al*., 2006).

The saponification number or saponification value is an important parameter used for the characterization and assessment of the quality of edible fats and oils. It is measured by the number of milligrams of NaOH/KOH needed to hydrolyse one gram of fat. The average molecular weight of the triacylglycerols in a sample is indicated by the saponification number. Lower molecular weight fatty acids are present when the saponification value is high. This indicates that the quality of the oil is good for making cooking salads (Pearson, 1975). The oil may not be edible and should only be used to make soap, oil-based ice cream, and shampoos if it has a low saponification value, which indicates that it contains a high proportion of higher fatty acids. The process of

saponification involves treating a neutral fat with an alkali to break it down into glycerol and fatty acids:

$$(R - COO)_3C_3H_5 + 3NaOH \rightarrow C_3H_5(OH)_3 + 3R - COONa \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.1)$$

Iodine value (IV) of a lipid is an indicator of a lipid's average degree of unsaturation the more the C=C double bonds there are in the lipid the higher the IV. Iodine value is measured by the number of grams of iodine absorbed per 100 grams of lipid. The "*Wijs* method" is one of the most widely used techniques for measuring a lipid's iodine value (AOAC 2005). After weighing and dissolving the lipid to be examined in an appropriate organic solvent, a known excess of iodine chloride is added. While the remainder of the ICl stays, some of it reacts with the unsaturated lipids' double bonds:

$$R - CH = CH - R + ICl_{excess} \rightarrow R - CHI - CHCI - R + ICl_{remaining} \ldots\ldots\ldots.. (2.2)$$

The amount of ICl left over after the reaction is complete is used to calculate how much ICI has reacted (ICl reacted = ICl - I~l.). To find out how much ICl is left, iodine is liberated from the remaining solution by adding excess potassium iodide. The concentration of iodine released is then measured by titrating with a sodium thiosulfate ($Na_2S_20_3$) solution in the presence of starch:

$$ICl_{remaining} + 2KI \rightarrow KCI + KI + I_2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. (2.3)$$

The reddish-brown color of iodine itself is frequently insufficiently strong to serve as a reliable indicator of the reaction's end-point. Since starch and iodine combine to form a deep blue molecular complex, starch is frequently used as an indicator. When the iodine-containing solution is first mixed with starch, it turns dark blue. A sodium thiosulfate solution with a known molarity is then used to titrate the solution. The solution remains blue as long as there is any iodine left in it; however, after all of the iodine has been transformed into iodide ions, it becomes colorless.

Peroxide value (PV) indicates the degree of lipid oxidation and hence its quality. It measures the peroxides formed during oxidation of a lipid. When compared to refined oils, unrefined vegetable oils exhibit higher PV. High PV values indicate high levels of oxidative rancidity in the oils as well as the lack or insufficiency of antioxidants such as propygadlate and butyl hydroxyl anisole are used to reduce rancidity (Kyari,

2008). A maximum allowed peroxide level of 10 mg equivalent of oxygen/kg of oils was established by the WHO/FAO (1994).

2.8 Fatty Acid Composition of the Avocado Oil

Lipids form a broad group of molecules including glycolipid (mainly triglycerides), free fatty acids FFA, sterol, phospholipids, and sphingolipids vitamins A and D (Gaydou *et al*., 1987; Meyer *et al*., 2008). Lipids from avocado fruits are essential nutrients and their study is of great importance. Total lipid content in an avocado fruit may reach up to 30% of the fruit by fresh weight basis (Ge *at al.*, 2017). The profiling of these lipids is an important process in determining their quality. Lipids which contain high unsaturated fatty acids are prone to oxidation leading to undesirable taste as well as compounds that are poisonous to human health (Bhattacharya *et al*., 2008). Manufacturers also rely on the lipid profile of any fruit to determine the processing conditions for their optimization.

Glycerol esterified to three fatty acid chains of different lengths and compositions, or as free fatty acids, make up fatty acids, which are found in lipids (Azizi *et al*., 2008). Each of these fatty acid chains has a unique chemical makeup and can be either saturated or unsaturated. Depending on the level of saturation or unsaturation, each chain is made up of different single- or double-bonded carbon and hydrogen atoms. Triglycerides are composed of mixed chains with heterogeneous structural comparisons between the chains.

$$\mathrm{HOCH_2CH(OH)CH_2OH + RCO_2H + R_2CO_2H + R_3CO_2H \rightarrow RCO_2CH_2CH(O_2CR_2)CH_2CO_2R_3 + 3H_2O}\ldots\ldots \quad (2.4)$$

R_1, R_2, R_3 in the above equation are different alkyl groups, as many kinds of triglycerides are known.

The chain lengths of the fatty acids in naturally occurring triglycerides vary, but only acids combining an even number of carbon atoms are in high percentage especially those containing 16, 18, or 20 carbon atoms.

Previous studies have shown that about 67% of the total lipids are unsaturated fatty acids, and 33% saturated fatty acids. The unsaturated fatty acids can be categorized into mono-unsaturated fatty acids (mainly oleic (C18:1) and palmitic (C16:1), poly-unsaturated fatty acids (linoleic (C18:2) and linolenic (C18:3) acids) and saturated

fatty acids (palmitoleic acid (C16:0) and stearic acid). Avocado also contains a small amount of arachidic acids (C20:0) and myristic acid (C14:0). Free fatty acids are present in avocado fruit in very small amount. (Doneltti and Terry 2014). Table 2.2 and 2.3 below shows the structure of some of the un-saturated and saturated fatty acids present in avocado oil and their structure respectively.

Table 2.2: Un-saturated fatty acids

Common Name	**systematic name**	**Molecular Formula**	**Structural Formula**	**omega - Series**
palmitoleic	9-hexadecenoic	$C_{16}H_{30}O_2$	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	ω-7
oleic	9-octadecenoic	$C_{18}H_{34}O_2$	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	ω-9
linoleic	9,12-octadecadienoic	$C_{18}H_{32}O_2$	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	ω-6
α-linolenic	9,12,15-octadecatrienoic	$C_{18}H_{30}O_2$	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	ω-3
γ-linolenic	6,9,12-octadecatrienoic	$C_{18}H_{30}O_2$	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_4COOH$	ω-6

Table 2.3: Saturated fatty acids

myristic acid	tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	C14:0
palmitic acid	hexadecanoic acid	$CH_3(CH_2)_{14}COOH$	C16:0
stearic acid	octadecanoic acid	$CH_3(CH_2)_{16}COOH$	C18:0
arachidic acid	eicosanoic acid	$CH_3(CH_2)_{18}COOH$	C20:0

One characteristic that determines the importance of unsaturated fatty acids in the body is their high percentage (Villa-Rodriguez *et al*., 2011; Dreher and Davenport, 2013). There are numerous metabolic processes that depend on these unsaturated fatty acids. Omega-3, omega-6, and omega-9 fatty acids, have a number of health advantages. However, the body cannot synthesize omega-3 and omega-6, which are necessary for blood pressure regulation, immune system function, and brain development, and must be obtained through diet. The body produces omega-9, a non-

essential fatty acid, when omega-3 and omega-6 are sufficient; otherwise, it must be taken. Omega-9 plays a key role in strengthening the body's immune system, lowering bad cholesterol, and encouraging heart health. (Villa-Rodríguez *et al.*, 2011; Dreher & Davenport, 2013; Donetti and Terry, 2014; Pedreschi *et al*., 2016). Avocado fruits are good source of these fatty acids important to human health and hence the need to investigate the oil composition in relation to growing area and cultivar.

Free fatty acids and triacylglycerol are not very volatile compounds (Morello *et al*., 2006). It is therefore difficult to analyse them using gas chromatography which require the sample to be vaporized in the instrument (Meyer *et al*., 2008). Free fatty acids and triacylglycerol are therefore derivatised first to increase their volatility before analysing them (Guerrero *et al.*, 2023). This is done by saponification using sodium hydroxide to reduce their molecular weight as follows;

$$(\mathrm{R-COO})_3\mathrm{C_3H_5} + 3\mathrm{NaOH} \rightarrow \mathrm{C_3H_5(OH)_3} + 3\mathrm{R-COONa} \quad \text{.....(2.5)}$$

Where:

$(R{-}COO)_3C_3H_5$ = Triglyceride

3NaOH = Three molecules of Sodium Hydroxide

$C_3H_5(OH)_3$ = Glycerol

$3R{-}COONa$ = Three molecules of Soap (sodium salt of fatty acid)

This reaction is crucial for the industrial and traditional production of soap.Saponification is followed by methylation using methanol to produce fatty acid methyl esters FAME which reduces polarity (Wang *et al*., 2020). Saponifying and methylation increases volatility of the lipids.

Acid-Catalyzed Transesterification of Triacylglycerides (TAGs) to Fatty Acid Methyl Esters (FAMEs) mechanism involves the triglyceride reacting with an alcohol (typically methanol for FAMEs) in the presence of an acid catalyst (like sulfuric acid, HCl, or sulfonic acids). The process occurs in a stepwise manner, replacing each fatty acid chain of the triglyceride with an alkyl group from the alcohol, forming FAMEs and glycerol.

The mechanism can be broken down in to the following steps:

Step 1: Protonation of the Carbonyl Oxygen

Step 2: Nucleophilic Attack by Methanol

Step 3: Proton Transfer within the Tetrahedral Intermediate

Step 4: Elimination of Fatty Acid Alkyl Group (as alcohol) and Formation of FAME

Step 5: Repetition for Remaining Ester Linkages

The newly formed diglyceride (still attached to the glycerol backbone) and then monoglyceride will undergo the same series of steps (Steps 1-4) with additional methanol molecules. Each step liberates another molecule of FAME and progressively converts the glyceride into a lower glyceride, eventually yielding glycerol.

The overall transformation is:

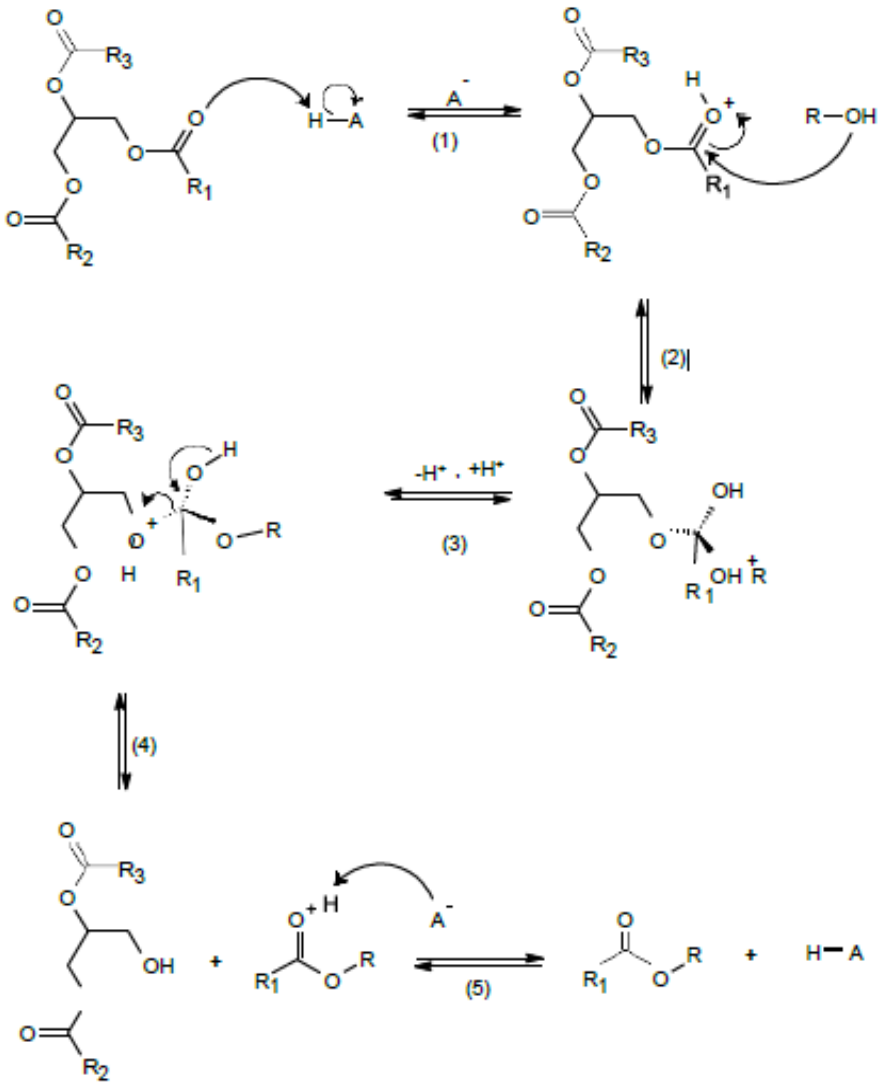
$$\text{TAG} + 3\,CH_3\text{OH}\,(\text{Acid Catalyst}, \text{Heat}) \rightarrow 3\,\text{FAMEs} + \text{Glycerol} \quad \text{(2.6)}$$

The scheme below shows homogeneous acid catalysed reaction mechanism for transesterification of triglyceride

Key:

A-H: Acid catalyst

R, R_1, R_2, R_3: Fatty acid alkyl groups (representing the hydrocarbon chain



The chain lengths of the fatty acids in naturally occurring triglycerides vary, but only acids. FAMEs are then analysed using GC and the fatty acids identified by comparing their retention time with those well characterized in literature. Their identity and the structure are established by connecting a GC and a mass spectrometer where the compounds are broken down into fragments characteristic of the compound and recorded in a spectrum.

Throughout the world there are many avocado cultivars with Hass having a better acceptance in the international market due to its high yield ad ability to withstand post-harvest processes. The amount and physicochemical properties of oil varies with the variety. Yanty *et al.* (2011) reported that Malaysian avocados possess completely different properties compared to the Hass imported from Australia. Similar studies were done in Brazil where 3 avocado cultivars obtained from one station and the study

reported that despite the avocados growing in the same environment, they yielded different amounts of oils (Galvao *et al*., 2014).

During GC-MS analysis on the three Indonesian avocado cultivars, different lipid profiles were reported as follows; MAB had 63 peaks, MAB 43 peaks and MAG 69 peaks (Indriyani *et al.,* 2016). Similar observations were made by Teng *et al.* (2016) who studied seven commercial avocado cultivars in Taiwan and Nair *et al.* (2018) who studied four cultivars in Kerala Nigeria. There are limited similar studies that have been carried out in Murang'a County.

Lu *et al.* (2008), studied Californian Hass avocado in four different locations and reported that GC-MS spectra shown different peaks. Carvalho *et al*. (2015) studied the relationship between fatty acid content and orchard altitude. The orchards altitude ranged between 1340m-2420m above sea level. It was reported that for an avocado, the cooler the area the higher the amount of lipids on fresh and dry matter basis. Oleic acid and linoleic acid were observed to significantly increase with rise in altitude. Similar observations were reported by Dixton *et al.* (2015) and Ferreyra *et al.* (2016). Avocados grown in different geographical areas show variation due to differences in climatic and soil conditions (Maitera *et al*., 2014). In all these studies oleic acid was suggested that it can be used as a marker of origin of avocado.

In all these studies local climatic conditions of the geographical growing area had a lot of influence on the lipid profile of the avocado. The study suggested that any research on the health benefits of avocado should consider the growing area of the fruit. The relationship between the lipid profiles with different avocado cultivars grown in Murang'a County has not been studied. The fatty acid profile of Hass avocado is the same but the amount of Palmitoleic acid increases with maturity (Lu *et al*., 2008). Linoleic acid was reported to increase during ripening of the avocado. (Eaks *et al.* (1990) and Pedreschi *et al.* (2014).

2.9 Elemental Composition of the Pulp and the Seed

Mineral elements composition evaluation is important in evaluating possible contribution of the seed and the pulp in improving sustainability of food and pharmaceutical industries. Most of the studies conducted focused on the fatty acids composition despite the avocado fruit been rich in minerals (Dreher & Davenport.,

2013; Vinha *et al*., 2013). Also, most of the studies done focused on the avocado pulp while the seed that accounts for 13-18% of the fresh fruit by mass has scarcely been studied. The seed is generally discarded during processing and this causes environmental pollution. Avocado seed contains more minerals than the pulp and their evaluation is important to determine their application in food and pharmaceutical industries (Ge *et al.,* 2017).

The elemental composition of the seed and the pulp vary greatly with geographical growing area and variety. Studies carried out in four commercial cultivars in Mexico reported that the amount of dry matter determines the amount of nutrients and not the size of the fruit or the amount of harvest (Hofman *et al.,* 2002). The report concluded that growers should expect significant difference in nutrients in different avocado cultivars. Growers should therefore determine the yield and the quality, by determining the nutrients removal, in any avocado cultivars. The evaluation of these elements would shed more light into possible application of the seed there by reducing pollution and improve the economy. Ge *et al.* (2017), compared the minerals in two china cultivars and reported that the seed contains more minerals than the pulp. The study also reported a difference between the cultivars. Similar observations were made on heavy metal analysis on selected fruits in Ethiopia where fruits shown different concentration of the elements depending on the area of origin (Kalagbor *et al*. 2014; Tegegne *et al.,* 2017).

2.10 Method of Analysis of avocado oil fatty acids and elemental composition

2.10.1 Gas Chromatography- Mass Spectrometry

The use of GC-MS is now widespread, and allows the design of more understandable spectral libraries and matching systems for quick and secure identification of compounds. GC/MS is an analytical technique that combines a gas chromatography and a mass spectrometer. Usually GC separates the compound that is eluting while mass spectrometer identifies and determines the structure of the fatty acids.

2.10.2 Gas Chromatography

Gas chromatography (GC) is a modern analytical technique of separating and analysing volatile substances without decomposing them. A typical GC system has an

injection port, a column packed with a stationary phase, a carrier gas control machine, ovens and heaters. Basic tool for analysing lipids. The main stationary phases are cyanopropyl siloxane, octadecyl groups, and sulphoxyl groups (Novák, 2021). The choice of the stationary phase depends on the polarity of the solute. A carrier gas (mobile phase) passes the sample under analysis through a column at varying speeds based on the sample's properties. Depending on its molecular weight, boiling point, and mode of interaction with the stationary phase, the sample is eluted at particular times (retention time). A detector that tracks retention times measures the amount of the material through the peak area or intensity on the corresponding chromatogram in addition to the time it leaves the column. Typically, the order in which the substances are released from the column determines their retention time (Poole, 2003).

2.10.3 Mass Spectrometry

The Mass Spectrometry (MS) is operated in selective ion-detection mode (quadrupole) to selections at very short mass intervals. This allows separate quantification of two components simultaneously eluted from GC, for example isotope counterparts (Reineccius, 2010). It should be emphasized that an MS detector is generally more sensitive than Flame Ionization Detection (FID) and this high ion selectivity is the key factor for achieving excellent results in the analysis of lipids extracts. The way a compound splits into fragments by beam of electrons energy is characteristics of its structure (Gerpen *et al.,* 2005).

2.10.4 Atomic Absorption Spectrometry

Using the absorption of optical radiation (light) by free atoms in the gaseous state, atomic absorption spectroscopy (AAS) is a spectro-analytical method for the quantitative determination of chemical elements. This technique is one of the best ways to detect minuscule amounts of a sample, like those that are present at one part per billion (ppb) levels (Mohammed. 2021). Different elements' atoms absorb light at different wavelengths. Light from that element is used to analyse a sample to determine whether it contains that element.

In AAS, a beam of electromagnetic radiation from excited atoms is passed through the vaporized sample after it has been atomized, or transformed into ground state free atoms in the vapour state. The atoms in the sample absorb a portion of the radiation.

Radiation absorption increases with the number of atoms in the vapour. The number of atoms determines how much light is absorbed. The population of ground and excited states can be explained by the Boltzmann equation;

$N1/No = (g1/go)\, e\, \Delta E/KT$……..... (2.7)

Where,

N_1 is the number of atoms in the excited state.

N_o is the number of atoms in the ground state.

g_1/g_o is the ratio of statistical weights for ground and excited states.

ΔE is the energy of excitation (=hV).

k = Boltzmann constant.

T=temperature in Kelvin

A calibration curve is constructed by running several samples of known concentration under the same conditions as the unknown (Figure 2.3). The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the concentration in the unknown sample.

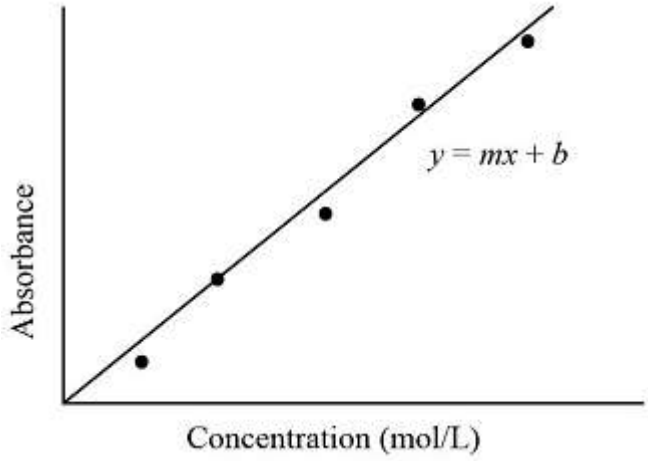


Figure 2.3: Graphical illustration of variation of concentration and absorbance.

According to Beer's Law, the number of absorbing species in a sample determines how much light is absorbed. The amount of light absorbed in the atomizer of atomic absorption is therefore directly proportional to the concentration of the analyte. The following is an expression for the combined Beer-Lambert law:

$I = I_0 e^{-\mu(x)}$……..... (2.8)

Where I is the intensity

I_0 is the initial intensity

x is the depth in meters

μ is the coefficient of absorption

Consequently, an atomic absorption spectrometer needs the following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed.

2.11 Research gaps addressed in the study

The amount and physicochemical properties of oil varies with the cultivar. Malaysian avocados possess completely different properties compared to the Hass imported from Australia (Yanty *et al.,* 2011). Similar studies were done by Galvao *et al*. (2014) in Brazil where 3 avocado cultivars were obtained from one station and the study reported that despite the avocados growing in the same environment, they yielded different amounts of oils. During GC-MS analysis on the varieties three Indonesian avocado varieties, the study reported different lipid profiles as follows: MAB had 63 peaks, MAB 43 peaks and MAG 69 peaks (Indriyani *et al.,* 2016).

Similar observations were made by Teng *et al.* (2016) who studied seven commercial avocado varieties in Taiwan, Nair *et al.* (2018) who studied four cultivars in Kerala Nigeria. Lu *et al.* (2008) studied Californian Hass avocado in four different locations and reported that GC-MS spectra shown different peaks. Carvalho *et al*. (2015), studied the relationship between fatty acid content and orchard altitude. The orchards altitude ranged between 1340m-2420m above sea level. It was reported that for an avocado the cooler the area the higher the amount of lipids on fresh and dry matter basis. Oleic acid and linoleic acid were observed to significantly increase with rise in altitude. Similar observations were reported by Dixton *et al.* (2015) and Ferreyra *et al.* (2016). According to Maitera *et al*. (2014) avocados grown in different geographical areas show variation due to differences in climatic and soil conditions.

In all these studies oleic acid was suggested that it can be used as a marker of origin of avocado. Muturi (1990), reported a great variation in avocado fat content of avocados obtained from Murang'a. In all these studies local climatic conditions of the geographical growing area had a lot of influence on the lipid profile of the avocado. The study suggested that any research on the health benefits of avocado should consider the growing area of the fruit. The relationship between the lipid profiles with different avocado varieties grown in Murang'a County has scantly been studied.

2.12 Summary

This literature review starts with the general topic of avocado production, then focuses on the specifics of production and cultivars in Murang'a County, Kenya. It then transitions to the nutritional value and quality determination of the avocado fruit, followed by a detailed analysis of its chemical composition, including moisture, oil, ash, and fiber content. The review pays special attention to the characteristics of avocado oil, covering its physicochemical properties and fatty acid composition. It also examines the elemental composition of both the pulp and the seed. Finally, it outlines the analytical methods used, specifically Gas Chromatography-Mass Spectrometry (GC-MS) and Atomic Absorption Spectrometry (AAS), and concludes by stating the research gaps addressed by the study.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Introduction

This chapter presents a comprehensive description of the materials, methods, and experimental procedures used in this research. Its primary goal is to provide a clear and reproducible account of the scientific approach, enabling others to validate the findings and replicate the experiments. The methodology systematically covers all aspects of the study, beginning with the detailed description of the study site and research design. It then outlines the procedures for sampling, sample preparation, and the use of specific reagents. Subsequent sections detail the analytical methods for proximate analysis, lipids extraction, and the determination of various physicochemical properties of the oil. The chapter concludes by describing the advanced techniques for determining fatty acid and elemental compositions, as well as the statistical methods used to analyse the data.

3.2 Study Site

The elevation of Murang'a County ranges from 914 to 3353 meters above sea level. The County is located between latitudes 0° 34' and 10° 7' South and longitudes 36° and 37° 27' East, with a total area of 2,558.8 km^2. Murang'a County has 6 ecological zones ranging from highest along the slopes of Aberdares where the economic activity is tea growing, low zones where coffee and dairy farming is the main activity and the flatter areas of Makuyu where coffee and pineapple growing is done by irrigation. The county has three different climatic regions Vis; climatic western zone, sub-tropical central zone and arid and semi-arid eastern zone (Figure 3.1).

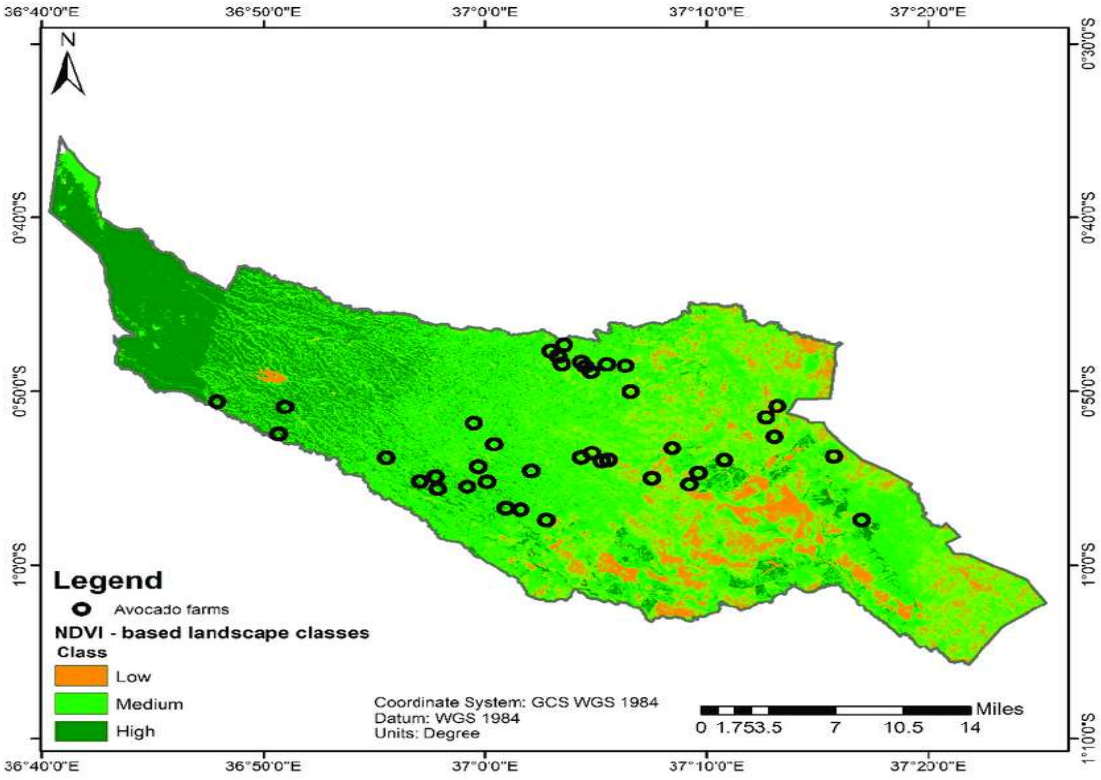


Figure 3.1: Distribution of avocado farms in Murang'a County, Kenya across three normalized difference vegetation index (NDVI)-based landscape classification (King'ori. 2022)

The county has varied climatic conditions and a number of different cultivar which can lead to great variation in quality and chemical composition of the avocado fruit. The fruits used in this study were obtained from selected farms at each of the three climatic zones. The farms from the upper zone were distributed above 2200m above sea level in Kanderendu area, Kangari ward, Kigumo constituency on coordinates (0º 47'S, 36º49'E). The farms from the middle zone were distributed between 1700m-2100m above sea level in Gatanga and Kihumbu-ini wards, Gatanga constituency on coordinates (0º 55'S, 36º57'E). The farms from the lower zone were distributed below 1600m above sea level in Ichagaki and Kamahuha areas, Maragua constituency on coordinates (0º 47'S, 36º49'E).

3.3 Research Design

This study involved extraction and profiling of oil from avocado fruits as well as elemental analysis of the fruit. The proximate (moisture, ash, oil content and dietary fibre), physicochemical (specific gravity, acid value, saponification value iodine value

and peroxide value), elemental (potassium, sodium, calcium, magnesium, zinc, iron and copper) and fatty acid composition of four avocado cultivars collected from three different climatic zones were determined.

3.4 Sampling and Sample Preparation

The samples were collected from Murang'a County by clustered sampling. The county was divided into three zones depending on the climate (i.e., upper, middle and lower zone). The zones were assigned numbers as follows; 1-upper zone, 2-middle zone and 3- lower zone. Figure 3.1, above shows the distribution of avocado farms in Murang'a County, central Kenya.

To maximize the representation of farms in Murang'a, small avocado farms (less than 0.4 hectares) were chosen and situated in several settlements. Four administrative wards were the focus of each vegetation productivity class. Within each ward, four farms, spaced 0.5 to 9 km apart and situated in distinct villages, were chosen. But in some wards where we had a few potential avocado farms or cultivars, at least two farms were chosen. A minimum of 5 trees under 30 years old on a farm were chosen for each cultivar. Mature fruits were identified and the samples labelled accordingly. The avocados collected were assigned random numbers according to variety and zone. A total of 10 fruits from each variety were collected and replication done. Three replicates for each variety were done.

The samples were sorted and kept for 4-7 days until they ripened. The ripe fruits were thoroughly washed with a lot of water to remove mud and any other materials on its surface. The fresh fruit were weighed then cut longitudinally to remove the seed. The peel was then be removed and the pulp cut into small pieces and crushed in a mortar. A 10 g of the sample was weighed and used for analysis.

3.5 Reagents and Chemicals

All the chemicals and reagents used in this study were of analytical grade except where stated otherwise. The solvents petroleum-ether (40°C-60°C), ethanol (99%), diethyl ether, tetra chloromethane, glacial acetic acid, Chloroform and *Wijs* solution were obtained from Scharlab.

Pure pellets of potassium hydroxide, sodium thiosulphate, sodium hydroxide and potassium iodide, concentrated Nitric (v) acid and concentrated hydrochloric acid used were standard laboratory reagents obtained from Sigma-Aldrich.

3.6 Lipids Extraction

The extraction of oil was done in a Soxhlet extractor using petroleum ether as the solvent as described by Manaf *et al.*, (2018) as follows; a homogenous sample of avocado pulp was dried in an oven for 24hrs at 70˚C then ground into fine powder. The fine powder was accurately to obtain 10g of the dried sample was mixed with 250ml of petroleum ether. The mixture was transferred into a Soxhlet extractor for 6-8 hours at 40˚C-60˚C. The extract was then evaporated in a rotary evaporator at 95˚C to evaporate the solvent. The lipids were then weighed and expressed in % w/w on the dried fruit weight basis.

3.7 Determination of Proximate Analysis

3.7.1 Determination of Moisture Content

In a clean, dry crucible (W1), two grams of the homogenous pulp sample were precisely weighed. To get a consistent weight, the crucible was oven-dried at 100°C to 105°C. After that, the crucible was left in the desiccator for half an hour to cool. It was weighed once again after cooling (W2). The following formula was used to get the % moisture:

$$\% \text{ Moisture } = (W1 - W2)/W0 \times 100 \quad \text{..... (3.1)}$$

Where:

W0 = Weight of sample (g), W1 = Initial weight of crucible + Sample (g), W2 = Final weight of crucible + Sample (g)

3.7.2 Determination of Ash Content

The weight of an empty crucible was measured and noted as (W0). The crucible was filled with two grammes of each homogenous pulp sample, and the weight was noted as (W1). For three hours, the crucible was heated to 550ºC in a furnace. After allowing the crucible to cool, the weight was noted as (W2). The following calculation was used to determine the percentage of ash content:

$$\% \text{ Ash content } = \ (W2 - W0)/(W1 - W0) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.2)$$

Where:

W0 = weight of empty crucible(g), W1 = weight of crucible + powdered sample(g), W2 = weight of crucible + ash sample(g). (AOAC, (2016)

3.7.3 Determination of Dietary Fiber

Using a Soxhlet, 2g of dried material pulp were precisely weighed, and the oil was extracted using petroleum ether for eight hours. A one-litre conical flask was filled with the dry residue that was free of fat. A beaker containing 200 ml of 2M sulphuric acid was heated to a boil. After transferring all of the boiling acid to the flask containing the fat-free material, the flask was instantly heated and attached to a reflux water condenser, causing its contents to boil in less than a minute. In order to prevent the material from sticking to the flask's sides and coming into contact with the acid, the flask rotated regularly. For precisely half an hour, the boiling was maintained. Following the removal of the flask, the liquid was filtered through fine linen (about 18 threads per centimetre) and rinsed with boiling water until it was no longer acidic to clear. A reflux condenser was used to bring the same amount of 2M sodium hydroxide solution to a boil. Using 200 ml of the boiling sodium hydroxide solution, the residue on the linen was rinsed into the flask. The reflux condenser was quickly attached to the flask, and it boiled for precisely half an hour. The mixture was promptly filtered through the filtering cloth once the flask was taken out. After being thoroughly cleaned with boiling water, the residue was moved to a Gooch crucible that had been prepared with a thin but compact layer of burned asbestos. After carefully washing the residue with hot water, 15 ml of 95 percent (by volume) ethyl alcohol were added. The Gooch crucible and its contents were weighed after being dried in an air oven at 105 ± 1°C until they reached a consistent mass. The Gooch crucible's contents were burned in a muffle furnace at 600 ± 20ºC until all of the carbonaceous material was gone. After cooling in a desiccator, the Gooch crucible with the ash was weighed once more. The formula was then used to determine the percentage fibre content.

$$\%\text{fibre} = \ (W1 - W2)/W0\text{x}100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.3)$$

Where:W_0= weight of sample, W_1= weight of dried sample,W_2= weight of ash sample.

3.8 Determination of Physicochemical Properties of Oil

The analyses of the physicochemical properties (specific gravity, acid value, saponification value, iodine value and peroxide value) of avocado oil was done as described by the AOAC methods 2005. (AOAC. 2005)

3.8.1 Determination of Specific Gravity

A clean and dry pycnometer was weighed and its weight recorded as x. The pycnometer was then filled with water, the temperature was adjusted to 25ºC in a water bath and weighed again. The weight was recorded as y. The pycnometer was emptied and dried. The pycnometer was then filled with oil and weighed again. The weight recorded as z. The formula below was used to calculate the Specific gravity

$$\text{Specific gravity} = (z - x) \div (y - x) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.4)$$

where x= mass of empty pycnometer, y = mass of pycnometer filled with distilled water, z = mass of pycnometer filled with oil.

3.8.2 Determination of Acid Value

Acid value was determine by the method described by Nasri *et al*. (2021), as follows; Two grams of oil were dissolved into solvent mixture of 25 ml of ethanol 99% and 25 ml of diethyl-ether, two drops of phenolphthalein added and the solution titrated with 0.5N potassium hydroxide until a pink end-point was reached. The acid value was determined by the formula

$$\text{Acid value} = 56.1V \times N \div w \ (\text{mg of KOH/g of oil}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.5)$$

where V= number of ml of the potassium hydroxide used, N =Normality of potassium hydroxide used and W = mass of oil used.

3.8.3 Determination of saponification value

The saponification value was determined by taking 1.0 g of oil sample in a conical flask to which 15 ml 1 N KOH (prepared by dissolving 56.0 g of KOH pellets in 400cm^3 of water and diluting it to 1000 ml in a clean volumetric flask) is added and 10 ml of distilled water and heated under a reserved condenser for 30–40 minutes to ensure that the sample fully dissolves.After that, sample was cooled, phenolphthalein

added and titrated with 0.5 M of HCl until a faint pink persisted for 15 seconds. 25 ml of KOH in which no oil is added was titrated with the standard HCl to determine the alkali originally added.

$$\text{Saponification value} = (VB - VW) \times 56.1N \text{ (mg KOH/g of oil)} \quad (3.6)$$

Where V_B is the volume of the Blank, V_W =volume of the sample N=1

3.8.4 Determination of Iodine Value

In order to determine the iodine value, 0.01N sodium thiosulphate was prepared by dissolving 25 g of sodium thiosulphate in freshly boiled water and the solution made up to 1000 ml in a volumetric flask. The solution was then standardized using 0.01N potassium dichromate solution. The normality of the sodium thiosulphate was then determined by the formula:

$$F = 20xF' \div (Vs - Vb) \quad (3.7)$$

Where F is the normality of sodium thiosulphate, F'=0.01, Vs is the volume of potassium dichromate used for the sample and Vb is the volume of the blank.

The 10% potassium iodide was prepared by dissolving 10g of KI in 100ml of water and starch solution prepared by dissolving 1.5g of starch in water and boiling it for 30 minutes.

Iodine value was determine by the method described by Nasri *et al*. (2021) as follows: 0.4g of the oil sample was accurately weighed into a conical flask. 20 ml of CCl_4 was added followed by 25cm^3 of *wijs* solution, then shaken vigorously for 30 seconds. The mixture was stored in the dark for 2.5 hours. A mixture of 20 ml of 10% KI with 125 ml of water was added. The solution was then titrated with 0.01N $Na_2S_2O_3$ using starch indicator until blue black coloration changed to colourless. Blank determination was done without oil.

$$IV = (B - S) \times N \times 12.69 \div W \text{ (g of iodine/100 g of oil)} \quad (3.8)$$

Where B is the quantity of sodium thiosulphate used for blank, S is the quantity of thiosulphate for sample, N is the normality of thiosulphate solution, W is the weight of the oil sample.

3.8.5 Determination of peroxide value (PV)

Peroxide value is a measure of peroxides contained in the oil. PV was determined by measuring iodine released from potassium iodide. A 0.3 g of oil sample was dissolved in a 10 ml of a solvent made by mixing glacial acetic acid and chloroform in the ratio 3:2. 1 ml of saturated KI (prepared by dissolving 100 g of KI in 70ml of distilled water) was added to the sample and the mixture immediately stoppered and store in the dark for 5 minutes. 20 ml of distilled water was then added and the amount of iodine liberated from KI by the oxidative action of peroxides present in the oil was determined by titration with 0.01N sodium thiosulphate using starch solution as an indicator.

$$\text{PV (meq of O2/kg of oil)} = (\text{Vs} - \text{Vb}) \times \text{F} \times \text{N} \times 1000 \div \text{W} \ldots\ldots\ldots\ldots\ldots (3.9)$$

Where Vb is the volume of sodium thiosulphate used for blank, W is the weight of sample, Vs is the volume of sodium thiosulphate consumed by the sample oil, N is the normality of standard sodium thiosulphate and F is the factor of 0.01N sodium thiosulphate.

3.9 Determination of Fatty Acid Composition

3.9.1 Derivatization of oil, identification and quantification of fatty acids

The oil extracted was converted into methyl esters as described by Yumeto *et al.* (2010), as follows; 8 ml of 95% methanolic hydrochloric acid was added to 0.2 g of oil. The mixture was then heated in a screw tight closed test tube for two hours at 85 ^{o}C. The mixture was then cooled under tap water to room temperature. The cooled mixture was transferred into a separating funnel. 4 ml of n-hexane added and shaken vigorously for 30 seconds. The mixture was allowed to stand and the hexane layer collected. The extraction was repeated one more time and hexane fractions combined. Four portions of water was added to the mixture to remove any acid. The solution was separated and dried using anhydrous sodium sulphate. A solution of fatty acid methyl esters was analysed using GC-MS as described by AOAC 2016 official method. The composition of the fatty acid of the avocado oil was identified analysis of their mass spectra and direct comparison with the Institute of Standards Technology libraries 98.1 (NIST) and Wiley Registry of Mass Spectral Data, 8th edition database of library of mass spectra, on the GC-MS equipment (NIST 2017).

3.9.2 GC-MS Instrumentation

For GC-MS fatty acid quantification was done at Murang'a university of technology analytical research laboratory using a single quadrupole mass spectrometer equipped with a gas chromatographic system (Agilent Technologies, Inc., 8860 GC system, CA, USA) GC-MS ISQ 7000 was used. Using an HP-5MS UI column (100 m ×0.250 mm × 0.25 mm, Agilent Technologies, Inc., CA, USA), FAMEs were separated chromatographically. As the carrier gas, helium (>99.999% purity) was utilised at a steady flow rate of 1.0 ml/min. The injection volume was set to 1.0 µL, and the injector temperature was set to 240 °C. The split mode with a 1:10 split ratio was used to insert all GC injections into the device. First, the temperature of the column was set to 125 °C and held for 1.5 minutes. Next, the temperature was increased at a rate of 10 °C min^{-1} to 180 °C, and held for 2 minutes. The temperature was then increased at a rate of 3 °C min^{-1} to 200 °C, then it was held for 20 minutes. Finally, 10 minutes were spent holding the temperature. The mass transfer line and ion source temperatures were 240°C and 230°C respectively. A temperature of 150 °C was chosen for the quadrupole. Electron impactionization (EI) in positive mode at 70 eV was used to ionise FAME standards and samples. The full scan mode was applied for qualitative analysis from 35–400 amu with a step size of 0.1 m/z, while the selected ion monitoring (SIM) mode was used for the quantitative analysis of FAMEs.

3.10 Determination of Elemental Composition

The pulp and the seed were analysed separately by the method described by Ge *et al.* (2017), with modification as follows; 5 g of the pulp and 5 g of the seed were dried at 105 **º**C in an oven to a constant mass and ground into fine powder then ashed at 450 ^{o}C. The 0.5 g of the ash was then digested using 5 ml of 65% HNO_3 for 1 hour at 140 **º**C. The mixture was placed in a water bath to evaporate the acid. The digested sample was dissolved in 25 ml distilled water and concentration of the essential elements determined by Atomic Absorption Spectrometer (AAS iCE 3000 series). Concentrated nitric acid was used as the blank control.

3.11 Statistical Analysis

The data obtained was statistically analysed using two-way analysis of variance (ANOVA) and Tukey's honestly significant difference test was done to evaluate the significance of differences between values at the level of $P<0.05$.

3.12 Summary

This chapter detailed the comprehensive methodology employed to characterize four different avocado cultivars grown in Murang'a County, Kenya. The study began with a clear description of the research site and the overall experimental design. Avocado fruit samples were collected and prepared through a meticulous process of sampling and sample preparation. The analysis was conducted using specific reagents and chemicals, as outlined.

The core of the methodology involved a series of analytical procedures. Lipids were extracted from the samples, followed by a proximate analysis to determine the moisture, ash, and crude fiber content. The physicochemical properties of the extracted oil were then investigated, with specific attention paid to specific gravity, acid value, saponification value, iodine value, and peroxide value. Furthermore, the fatty acid composition of the oils was determined using Gas Chromatography-Mass Spectrometry (GC-MS) instrumentation. The chapter also outlined the methods for determining the elemental composition of the avocado samples. Finally, the chapter concluded with a description of the statistical analysis techniques used to interpret the data, ensuring the validity and reliability of the research findings.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Introduction

This chapter systematically presents and interprets the findings from the experimental procedures detailed in Chapter Three. The results are organized to address the study's objectives, beginning with the proximate composition of the samples, which includes moisture, ash, and lipid content. This is followed by a detailed discussion of the physicochemical properties of the extracted oils, such as specific gravity, acid value, iodine value, saponification value, and peroxide value. The chapter concludes with the presentation and analysis of the elemental composition, focusing on key minerals like potassium, sodium, calcium, magnesium, iron, zinc, and copper. Each section provides a comprehensive discussion of the data, relating the findings to existing literature and highlighting their significance.

4.2 Proximate Composition

Four different avocado cultivars grown in different ecological zones in Murang'a County were analysed to determine their proximate composition (moisture, ash, lipid and dietary fibre content). The results are presented in sections below.

4.2.1 Moisture Content

Moisture content was determined on the edible part of the fruit as described in section 3.5.1. The results show the weights were monitored from time to time until a constant weight was reached. The results for moisture content was as shown in the Table 4.1

Table 4.1: Percentage Moisture content of the pulp of avocado cultivars from different zones

Zone	Hass	Pinkerton	Giant	Fuerte
1	69.91 ±0.74^{bc}	84.31 ±1.65^{i}	76.97 ±1.57^{def}	67.63 ±0.88^{b}
2	62.44 ±1.27^{a}	77.98 ±1.85^{defg}	69.96 ±0.61^{bc}	78.95 ±1.45^{efgh}
3	78.78 ±0.61^{de}	76.17 ±0.14^{efgh}	75.09 ±0.36^{d}	59.30 ±0.53^{a}

Where: - Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference test

The moisture content ranged between 59.30% for Fuerte grown in the zone 3 to 84.31% for Pinkerton grown in zone 1. The Hass avocado moisture content ranged from 62.44% to 78.78%. For Pinkerton the highest moisture content was reported in

avocado collected from zone 1 (84.31%) and the lowest in zone 3 (76.17%). For Giant avocado the highest moisture content was reported in zone 1 and the lowest in zone 2. Fuerte avocado had the lowest moisture content of 59.30% in zone 3 and the highest moisture content in zone 2 (78.95%). The moisture content was significantly different among the cultivars and among the zones at $p< 0.05$.

Moisture content decreases with increase in maturity of the fruit (Manaf *et al*., 2018). From the results, the Hass fruits collected from zone 2 were more mature than those collected from zone 1 and zone 3. For Pinkerton, the fruits collected from zone 1were less mature than those collected from zone 2 and zone 3. Giant fruits were more mature in zone 2 while Fuerte was more mature in zone 3. The difference in the moisture content may be due to difficulties in determining the maturity of the fruits from the field. However, this does not mean the fruits grown in the same area could not attain the same maturity level (*Carvalho et al. 2015*). According to agriculture and food authority, horticultural crops directorate Hass and Fuerte avocados harvesting guide, fruits should attain at least 20% dry matter content and not less than 8% oil content. In this line all the fruits collected from the farms met these criteria except Pinkerton grown in cold areas with a zone 1 content of about 16%.

Previous studies reported moisture content of 64.73% (Maitera *et al.*, 2014), 51.69% (Nwaokobia *et al*., 2018) in avocado pulp. Percentage moisture content reported in this project are similar to those reported in other regions of the world. Hass avocado collected from orchards of located at different altitudes had moisture content some of which were significantly different while others were not. At 2420 m above sea level (asl) and 1340 m asl, the moisture content was not significantly different at 69.3% and 69.9% respectively. The moisture content was also not significantly different at 2248 m asl (71.7%) and at 1770 m asl (71.8%). The difference was due to different maturity stages of the fruits (Carvalho *et al., 2015*). The moisture content cultivar of Fortuna (72.2%), Collison (76.4%) and Barker (75%) were significantly different (Galvao *et al*., 2014). The moisture content of the pulp, seed and peel of the same cultivar was also significantly different (Galvao *et al*., 2014). This report agrees with other reports indicating that avocado fruits contain high moisture content and that the moisture content vary with maturity, cultivar, part of the fruit analyzed and geographical growing environment.

4.2.2 Ash Content

The ash content of the pulp was determined and the data obtained was as follows;

Table 4.2: Ash content of the pulp of avocado cultivars from different zones

Zone	Ash content %			
	Hass	**Pinkerton**	**Giant**	**Fuerte**
1	0.97 ± 0.01^{b}	1.09 ± 0.10^{bc}	1.47 ± 0.08^{ef}	1.06 ± 0.09^{bc}
2	0.63 ± 0.01^{a}	1.34 ± 0.05^{de}	1.54 ± 0.06^{f}	1.04 ± 0.05^{c}
3	1.28 ± 0.04^{d}	1.55 ± 0.06^{f}	1.59 ± 0.03^{f}	1.47 ± 0.10^{ef}

Where: - Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference (HSD = 0.19) test

Ash content is the measure of total mineral present within a food. The ash content of the pulp of avocado cultivars from different ecological zones was determined and the data obtained is given in Table 4.2. Ash content range between 0.63% and 1.59%. Giant cultivar had the highest ash content in all zones with a mean of 1.53% followed by Pinkerton with a mean of 1.32% while Fuerte and Hass had ash content of 1.19% and 0.96% respectively. This indicated that Giant cultivar has a higher mineral uptake among the four cultivars studied. Pinkerton and Giant showed a linear relationship in ash content with decreased in altitude. Hass and Fuerte percentage ash content was lowest in zone 2 and highest in zone 3.

Figure 4.1 illustrates the variation of average percentage ash content per zone. It was clear that zone 3 had the highest average of 1.47% followed by zone 1 with an average of 1.15% while zone 2 had the least average of 1.14%. The ash content was more varied in zone 2 and less varied in zone 3. There was significant difference in the ash content between the cultivars ($p<0.05$) and between the geographical growing environment ($p<0.05$).

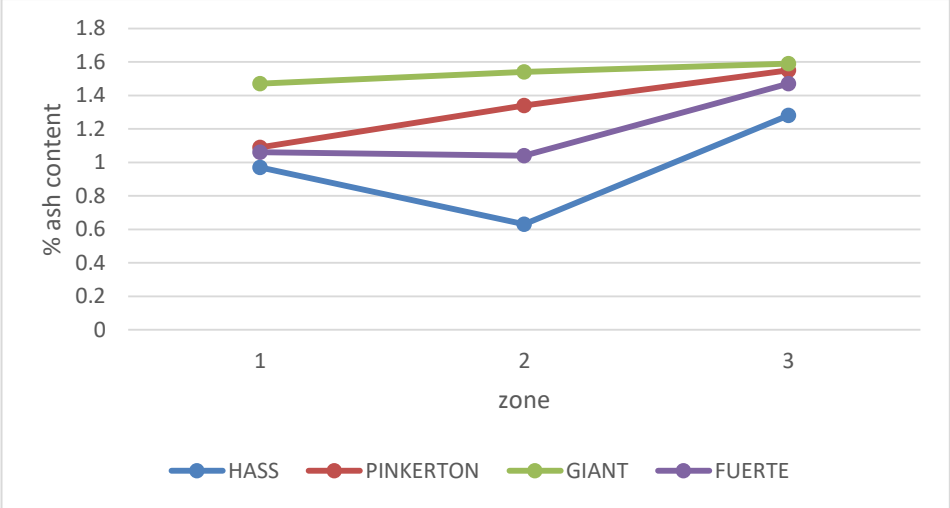


Figure 4.1: Ash content of avocado cultivars per zone

Similar observations were in a study by Nwaokobia *et al.* (2018), where avocado had 1.02% ash content and in a study by Nair *et al.* (2018), where Pollock, Kallar round, purple hybrid and Fuerte avocados had 1.22%, 1.16%, 1.20%and 1.02% ash content respectively. The ash content was higher than was reported by Ge *et al*. (2017), in RN-7 cultivar (0.52%). the ash content differs according to cultivar (Nair *et al.* (2018), geographical growing environment (Grembecka *et al*., 2013; Maitera *et al*., 2014) and the part of the fruit analyzed (Rodríguez-Carpena *et al*., 2011; Vinha *et al*., 2013; Galvão *et al*., 2014).

4.2.3 Lipid Content

This study investigated the influence of the cultivar and the growing climatic conditions on the lipid content of avocados. The results are as shown in Figure 4.2.

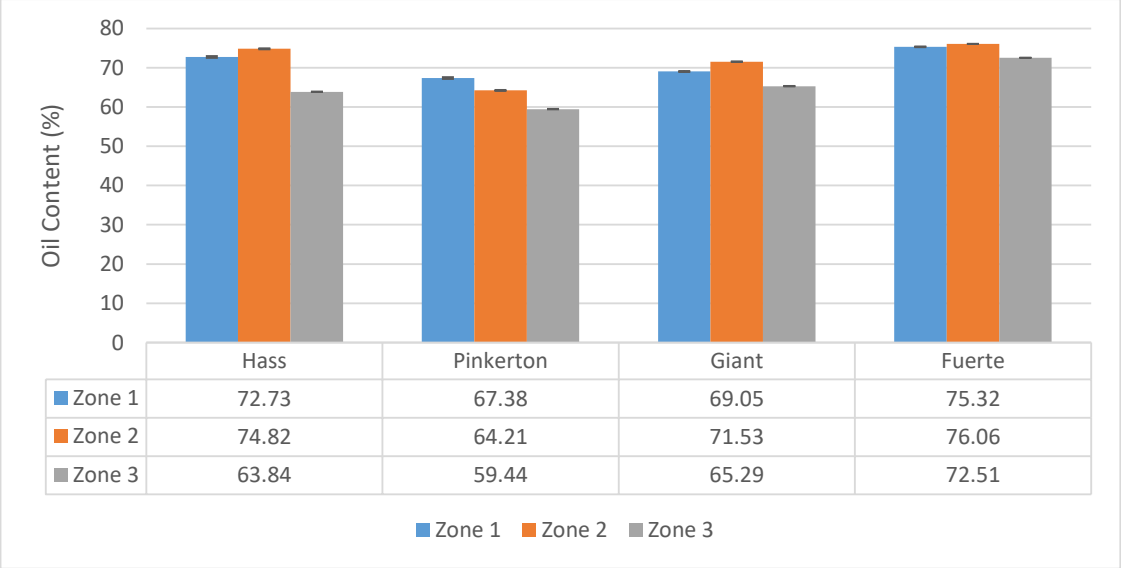


Figure 4.2: Avocado oil yield per cultivar per zone

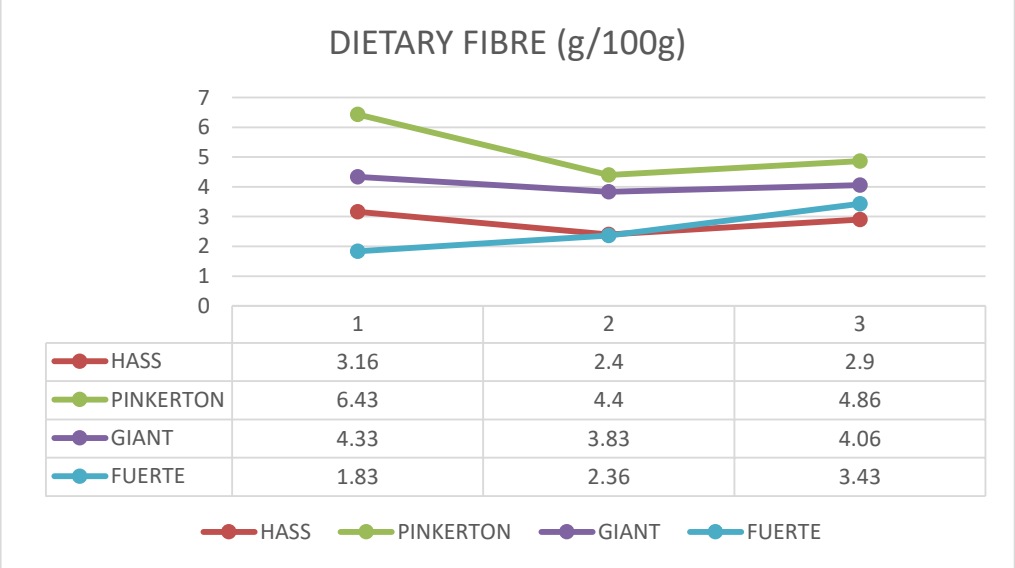
The oil content of avocado grown in Murang'a County ranged between 59.44% - 76.06% on dry weight basis. Fuerte avocado had the highest oil yield in zone 2 (76.06%) and lowest in zone 3 (72.51%). Pinkerton had the highest oil content in zone 1 (67.38%) and the lowest in zone 3 (59.44%). Hass and giant had the highest oil content in zone 2 (74.82% and 71.53% respectively) and lowest in zone 3 (63.84% and 65.29% respectively). The study reported the highest mean percentage oil yield in Fuerte avocado (74.63%), followed by Hass (70.46%), then Giant (68.62%), and the lowest in Pinkerton avocado (63.68%). The results showed the highest average of oil content of the four cultivars in zone 2 at 71.625% followed by zone 1 at 71.075% and the lowest oil content in zone 3 at 65.23%. This shows that the avocado cultivar and the geographical growing environment has an influence on the percentage oil content of the avocado fruits. There was significant difference on the oil yield among the cultivars and between the geographical growing environments at $P \leq 0.05$.

The percentage oil yields reported in this study are higher than those reported earlier in Indonesia of 36.18%-47.52% (*Manaf et al. 2018)*, Nigeria 36.4% (*Maitera et al. 2014*) and Colombia of 18.9% (Carvalho et al 2015). The percentage oil yield was observed to be lower in all cultivars in zone 3 (low altitudes). Similar observation was obtained by Carvalho *et al*. (2015) where Hass avocado was harvested from orchards at different altitudes. At 2420 m asl, 1900 m asl and 1510 m asl Hass avocado yielded 18.9, 14.1 and 7.8% oil content respectively (Carvalho *et al*., 2015).

The quantity of oil in avocado fruit is of great significance to individuals and industrialists. Those interested in oil prefer the fruit with high oil content while those who are diet and cholesterol conscious prefer fruits with lower oil content. Several factors determine the quantity of oil from avocado: these are cultivar, growing climatic conditions, part of the fruit and maturation (Galvão *et al*., 2014).

4.2.4 Dietary Fibre Content

The study found that the dietary fibre content was highest in zone 1 for Hass (3.16%), Pinkerton (6.43%) and Giant (4.33%) followed by zone 3 and lowest in zone 2 (Figure 4.3). For Fuerte the dietary fibre was lowest in zone 1(1.83%). The dietary fibre increased with decrease in altitude. For Hass the highest dietary fibre value is obtained from zone 1 (DF=3.16%) and lowest in zone 2(DF=2.4%) giving a range of 0.76%. The range in Pinkerton cultivar between the three zones was 1.57, in Giant the range was 0.5 while in Fuerte it was 1.6%. The highest mean was in Pinkerton (5.23%), followed by Giant (4.07%), Hass (2.82%) and the lowest average was from Fuerte (2.54%). This shows that growing climatic conditions significantly determines the amount of dietary fibre in different avocados cultivars. A high range of 4.6% was observed between cultivars in zone1 followed by zone 2 while it was least in zone 3. This shows that different avocado cultivars grown in the same environmental conditions have significantly different percentage dietary fibre.



	1	2	3
HASS	3.16	2.4	2.9
PINKERTON	6.43	4.4	4.86
GIANT	4.33	3.83	4.06
FUERTE	1.83	2.36	3.43

Figure 4.3: Dietary fibre content for avocado cultivar per zones.

The dietary fibre values obtained are higher than those obtained by Galvao *et al.*, 2014 from the pulp of the Fortuna (1.6g/100g), Collinson (1.3g/100g) and Barker cultivars (1.0g/100g). The total dietary fiber content of the avocado pulp in all cultivars was higher than the value of 0.4 g/100g reported by Moreno *et al*. (2003), and lower than the value of 6.3g/100g reported by Gondim *et al*. (2005). Given that they contain a significant amount of dietary fibre, a nutrient associated with better gut health, avocados can undoubtedly be a cornerstone of gut health. One avocado has about 13.5g of fibre, which about half of the 28 to 34g is recommended daily by the 2020–2025 Dietary Guidelines for Americans, according to the USDA.

Table 4.3: ANOVA analysis for proximate composition of avocado pulp

Parameter	***Source of Variation***	***SS***	***df***	***MS***	***F***	***P-value***
Moisture content	Zone	45.15048	2	22.57524	18.17853	1.56E-05
	Cultivar	621.2657	3	207.0886	166.7564	3.34E-16
Ash content	Zone	0.885551	2	0.442776	103.1857	1.63E-12
	Cultivar	1.561264	3	0.520421	121.2805	1.23E-14
Lipid content	Zone	301.3522	2	150.6761	919.6576	2.08E-23
	Cultivar	556.3896	3	185.4632	1131.982	5.73E-26
Dietary fibre content	Zone	3.260556	2	1.630278	21.26449	4.86E-06
	Cultivar	41.36306	3	13.78769	179.8394	1.41E-16

4.3 Physicochemical Properties of Oils Extracted from Different Avocado Cultivars.

Many government rules require that the purity of fats and oils be mentioned on food packaging, which makes it a crucial metric to measure. When it comes to the lipid's use in different consumer goods, its physical characteristics are crucial. The following physicochemical characteristics of oil were examined in the study: specific gravity, acid value, saponification value, peroxide value, and iodine value.

4.3.1 Specific Gravity

The ratio of an oil's density to the density of an equivalent amount of water is known as its specific gravity. Water and oil must have the same temperature because temperature has an effect on density. The chemical makeup of the oil affects its

specific gravity. Oil with a greater specific gravity is thought to contain aromatic chemicals, whereas oil with a lower specific gravity is thought to contain saturated compounds. Table4.4 below shows the data on specific gravity of pulp oils from the four varieties. The results obtained from this study indicates that specific gravity ranged from 0.85 to 0.92. This indicates that avocado oil is less dense than water. Hass, Pinkerton and Giant cultivars grown in higher altitude area with relatively cold weather conditions had a higher specific gravity.

Table 4.4: Specific gravity of avocado oil per cultivar per zone.

	Zone 1	**Zone 2**	**Zone 3**
Fuerte	0.86 ± 0.001^{abc}	0.88 ± 0.009^{bcdef}	0.89 ± 0.005^{defgh}
Giant	0.91 ± 0.001^{hijk}	0.89 ± 0.003^{defg}	0.88 ± 0.002^{bcde}
Hass	0.91 ± 0.013^{efghij}	0.85 ± 0.002^{a}	0.87 ± 0.001^{abcd}
Pinkerton	0.92 ± 0.023^{ijk}	0.89 ± 0.012^{defghi}	0.85 ± 0.003^{ab}

Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference test.

The results in this study can be compared with those obtained in Nigeria 0.86 (Ogbuagu *et al*., 2020), 0.91, 0.93 and 0.91 in the cultivars of Fortuna, Collinson and Barker respectively (Galvao *et al.,* 2014) and 0.92 reported in Gembu (Maitera *et al*., 2014).

4.3.2 Acid Value

The acid value is the number of milligrams of potassium hydroxide required to neutralise the free acid in 1.0g of the material. The acid value quantifies how triacylglycrols break down into free fatty acids, which is detrimental. Any substance that oxidises gets spoilt and loses its quality; oxidation also increases the substance's acidity. Similarly, the acid value of oxidised oil is high. The acid value of a good oil should be less than 0.1. It is best to interpret the increase in acid value as an indication of oil oxidation, which can lead to corrosion as well as the formation of sludge and gum.

The acid value characteristics of pulp oils from the four varieties are shown in Table 4.5. The results indicate that the acid values range from 0.19 to 0.98. Fuerte cultivar had the highest acid value in all the zones while Hass has the lowest.

Table 4.5: Acid value (mg KOH/g oil) of avocado oil per cultivar per zone.

	Zone 1	Zone 2	Zone 3
Fuerte	0.98 ± 0.28^{abc}	0.81 ± 0.11^{a}	0.65 ± 0.81^{ab}
Giant	0.51 ± 0.04^{abcde}	0.51 ± 0.20^{abcd}	0.33 ± 0.40^{ab}
Hass	0.32 ± 0.08^{abcde}	0.19 ± 0.81^{abcde}	0.23 ± 0.17^{abc}
Pinkerton	0.51 ± 0.11^{fgh}	0.37 ± 0.03^{fg}	0.23 ± 0.23^{cdf}

Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference test.

These acid values were significantly different between the cultivars and between geographical growing environments. These values are similar to the values were 0.49, 0.51, and 0.54 for the pulp oil of the Fortuna, Collinson, and Barker cultivars, respectively (Galvao *et al*., 2014). The acid values were lower than 0.19 obtained in Nigeria (Maitera *et al*., 2014).

4.3.3 Iodine Value

The iodine value is a frequently used indicator to evaluate the level of oil saturation or unsaturation. The iodine value is the mass of iodine in grams that is consumed by 100 grams of a chemical compound. Iodine levels are commonly used to estimate the degree of unsaturation in fats, oils, and waxes. Double bonds, which are the most common kind of unsaturation in fatty acids, react aggressively with halogens, such as iodine in this case. As a result, the higher the iodine value, the more unsaturated the fat. Data on a few iodine value attributes of the four types of pulp oils are displayed in Table 4.6.

Table 4.6: Iodine values (g/100g) of avocado oil per cultivar per zone

	Zone 1	Zone 2	Zone 3
Fuerte	95 ± 3.00^{ghij}	89.6 ± 4.72^{gh}	92.6 ± 6.80^{ghi}
Giant	71 ± 1.73^{abcdef}	67 ± 3.46^{abc}	69.3 ± 5.50^{abcd}
Hass	106 ± 1.73^{hijk}	107.3 ± 8.50^{hijkl}	79.6 ± 6.80^{cdefg}
Pinkerton	70.3 ± 6.65^{abcde}	58.3 ± 11.9^{ab}	54.3 ± 5.13^{a}

Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference test.

The iodine value of the oils ranged from 54.3 to 107.3. These values were within the range reported in other parts of world. (Yanty *et al*., 2011, Maitera *et al*., 2014, Indriyani *et al*., 2016). The iodine values of avocado collected in zone 1 are higher than those collected in zone 2 and 3. This indicates that there is higher concentration of unsaturated fatty acids for avocado grown in higher altitude. Hass had higher iodine value compared to the other cultivars in this study. The higher iodine values indicate the Hass avocado oil contains more unsaturated fatty acid (Indiyani *et al*., 2014). There was significant difference in the iodine values observed between the cultivars and the zones.

4.3.4 Saponification Value

The saponification value (SV) is a useful tool for determining the average relative molecular mass of oils and fats. All of the fatty acids that are present in the sample as triglycerides are measured for average molecular weight, commonly referred to as chain length. Alkali is consumed by glycerides, free fatty acids, and other compounds related to ester, such as lactones. Triglycerides, diglycerides, and monoglycerides are all forms of glycerides. The remaining alkali is titrated against a standard hydrochloric acid solution at the end of the procedure. The saponification value is the quantity of alkali (in this case, milligrams of potassium hydroxide) required to saponify a given quantity of fat or oil. Data on some saponification values of pulp oils from the four types are displayed in Table 4.7.

Table 4.7: Saponification value (g/100g) of avocado oil per cultivar per zone

	Zone 1	**Zone 2**	**Zone 3**
Fuerte	145.86 ±5.61^{efg}	142.12 ±3.23^{def}	158.95 ±3.23^{ghi}
Giant	127.16 ±3.23^{ab}	123.42 ±5.61^{a}	138.38 ±8.56^{cde}
Hass	162.69 ±5.61^{hij}	127.16 ±6.47^{abc}	130.9 ±3.23^{abcd}
Pinkerton	158.3 ±19.43^{gh}	187 ±3.23^{k}	166.43 ±3.23^{hij}

Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference test.

The study reported a saponification value range of 123.42 to 187. On average avocado fruits collected in zone 1 had a higher saponification value (151.00) while zone 2 had

the lowest saponification value (144.92). There were significant differences between the cultivars and zones. The differences observed are due to geographical growing environment and cultivar.

4.3.5 Peroxide Value

A freshness indicator for edible oils is the peroxide value (PV), which is established via oxidative degradation. The amount of molecular oxygen that first oxidises the hydroxyl groups in unsaturated fats in oils to create hydro peroxides and peroxides is known as PV in chemistry. For this amount of oil, milliequivalents O_2/kg (mEq O_2/kg) are commonly used as the unit of measurement. Rancid or spoilt oils have peroxide values greater than 30 mEq O_2/kg, while fresh oils have peroxide values less than around 10 mEq O_2/kg. Additionally, quantities of peroxide as high as 100 mEq O_2/kg have been linked to food poisoning cases. Data on certain peroxide values of pulp oils from the four types are displayed in Table 4.8.

Table 4.8: Peroxide value (meq O_2/kg fat) of avocado oil per cultivar per zone

Cultivar	Zone 1	Zone 2	Zone 3
Fuerte	2.13 ± 0.06^{jkl}	1.26 ± 0.04^{bcdef}	2.3 ± 0.17^{lm}
Giant	1.96 ± 0.11^{hij}	1.03 ± 0.03^{abc}	1.87 ± 0.07^{hi}
Hass	1.09 ± 0.12^{abcd}	1.82 ± 0.03^{h}	1.43 ± 0.06^{fg}
Pinkerton	0.90 ± 0.07^{a}	0.99 ± 0.13^{ab}	1.14 ± 0.13^{abcde}

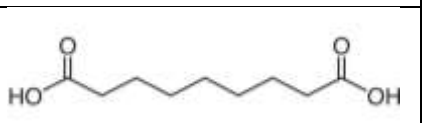
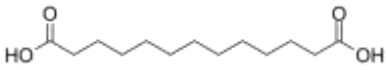
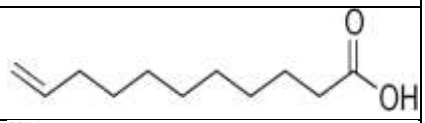
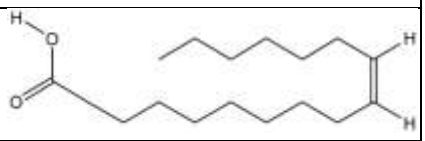
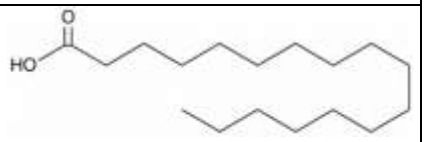
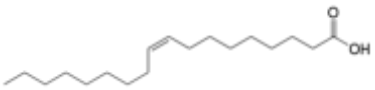
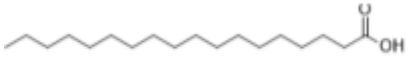
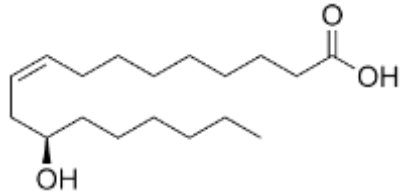
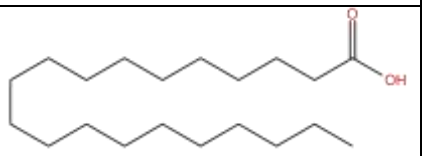
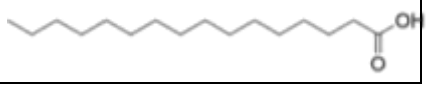
Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference test

The peroxide values ranged from 0.9 to 2.3. Fuerte avocado oil had relatively higher peroxide value compared to the other cultivars, indicating the oil is unstable against oxidation. While Pinkerton had the lowest indicating the oil is stable against oxidation. These values are similar to those reported in other studies ranging from 2.05 to 2.6 (Galvao *et al.*, 2014). The values reported varied with geographical growing environment, cultivar and soil composition.

4.4 Fatty Acid Composition of Oil

Fatty acid composition of the avocado oil was done analyzed using gas chromatography coupled with mass spectrometer (GC-MS), after converting the oils to the respective fatty acid methyl esters (FAME). A total of 10 different fatty acids were identified and quantified (Table 4.9). The fatty acids present were identified to be 9-hexadecanoic acid, 9-octadecanoic (z), stearic acid, ricinoleic acid, nanonedioic acid, tridecanedioic acid, palmitic acid, eicosanoic acid, undecylenic acid and dodecanoic acid.

Table 4.9: Fatty acids in avocado oil samples

Fatty acid	Common names	Retention Time (minutes)	Chemical structure
Nanonedioic acid	Azelaic acid	3.02	
Tridecanedioic acid	Brassylic acid	4.13	
10-Undecylenic acid	Undecylenic acid	4.50	
9-Hexadecenoic acid	Palmitoleic (C16:1)	6.92	
Dodecanoic acid	Lauric acid (12:0)	7.00	
Heptadecanoic acid	Heptadecanoic acid (17:0)	8.30	
9-Octadecenoic acid (z)	Oleic (c18:1)	9.40	
Octadecanoic acid	Stearic (c18:0)	10.05	
12-hydroxy oleic	Ricinoleic acid	13.23	
Eicosanoic acid	Eicosanoic (c20:0)	13.80	
Hexadecanoic acid	Palmitic (c16:0)	18.35	

The mass spectrum of 9-Octadecenoic (oleic) acid methyl ester (Figure 4.4) showed a molecular ion peak at m/z = 296 $[M]^+$ corresponding to the molecular formula $C_{19}H_{36}O_2$ and other diagnostic peaks at m/z values at 264 (M-32), 222 (M-74), 180

(M-116) and 101 (M-113) which are consistent with data previously reported for the compound (Abdel *et al*., 2019).

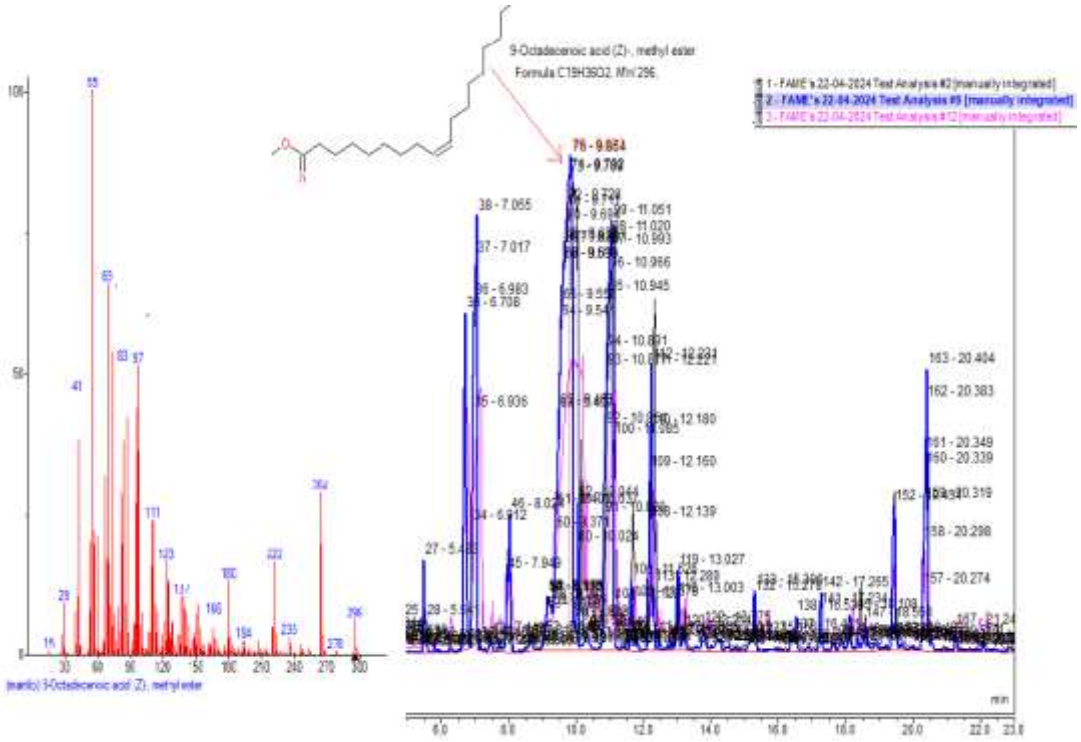


Figure 4.4: Mass spectrum and GC chromatogram for 9-Octadecenoic acid (z) methyl ester

Mass spectrum of Octadecanoic (stearic) acid methyl ester (Figure 4.5) showed a molecular ion peak at m/z = 298 $[M]^+$ corresponding to the molecular formula $C_{19}H_{38}O_2$ and other diagnostic peaks at m/z values at 267 (M-31), 255 (M-43), 199 (M-99) and 143 (M-155) which are consistent with MS data previously reported for the compound (Abdel *et al*., 2019).

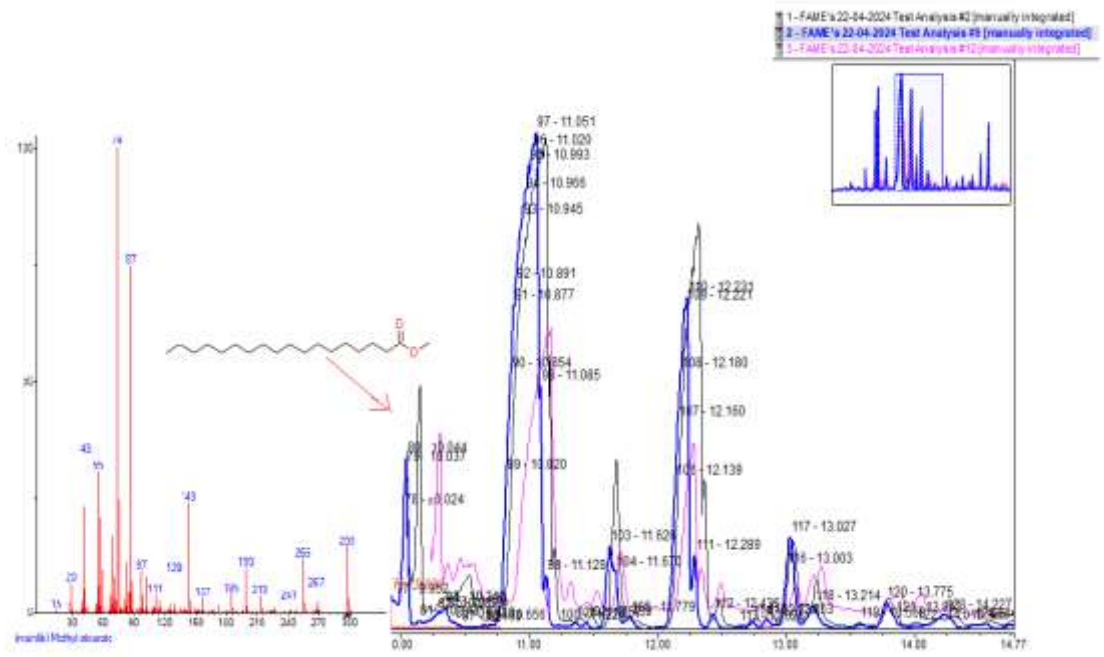


Figure 4.5: Mass spectrum and GC chromatogram for methyl stearate

The mass spectrum (Figure 4.6) of Hexadecanoic (palmitic) acid methyl ester showed a molecular peak at m/z = 270 corresponding to the molecular formula $C_{17}H_{34}O_2$ and other diagnostic fragment peaks at m/z values at 234 $[M\text{-}MeO]^+$, 143$[M\text{-}C_7H_{15}]^+$ and 87$[C_4H_7O_2]^+$ which were consistent with MS data previously reported for the compound (Abdel *et al*. 2019).

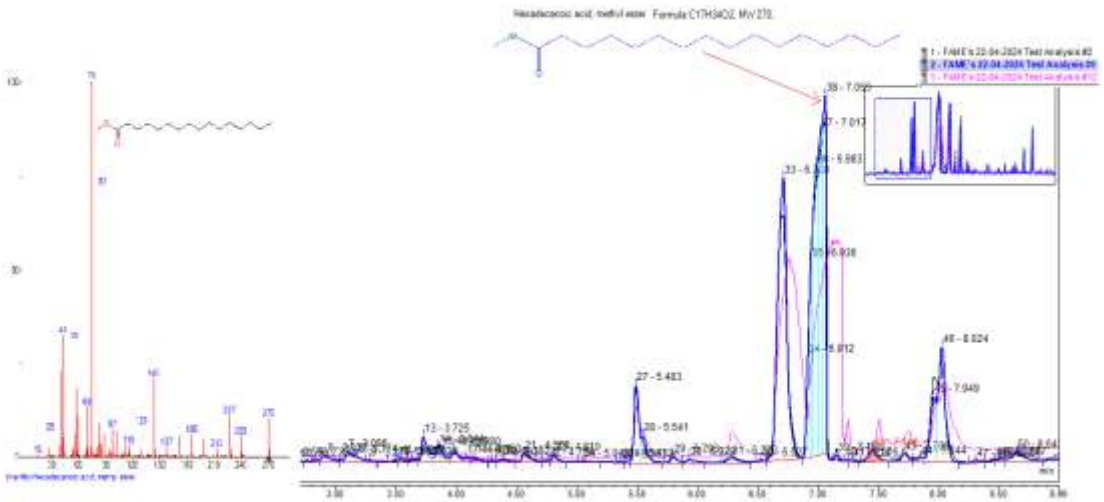


Figure 4.6: Mass spectrum and GC chromatogram for hexadecanoic methyl ester

Four fatty acids were present in all cultivars while seven fatty acids were present in one or more cultivars (table 13). The most dominant fatty acid was oleic acid whose concentration ranged from 66.32% to 78.67% in all the cultivars from all the three zones. The second most dominant fatty acid was by palmitoleic acid whose concentration ranged from 12.49% to 27.62%. Fuerte grown in zone 1 had the highest percentage of oleic acid 78.62% followed by Giant grown in zone 1 (78.52%). Hass grown in zone 1 had 78.46% while Pinkerton had 73.15%.

Table 4.10: Fatty acids composition per cultivar in the various zones

	Fatty acid composition (%)											
Cultivar	**H 1**	**H 2**	**H 3**	**P1**	**P2**	**P3**	**G1**	**G2**	**G3**	**F1**	**F2**	**F3**
Dodecanoic acid	ND	ND	ND	ND	ND	ND	0.04	ND	ND	ND	ND	ND
Palmitic acid	ND	0.79	ND	2.21	0.43	ND	ND	0.34	1.03	ND	0.21	ND
9-hexadecanoic acid	17.68	22.75	27.62	20.41	16.31	23.47	24.99	18.32	12.49	17	20.71	23.6
Heptadecanoic acid	ND	ND	ND	ND	ND	ND	0.05	0.04	0.07	ND	ND	ND
Stearic acid	2.49	2.54	1.7	1.5	2.4	1.91	5.36	2.43	3.4	2.95	1.55	1.94
9-octadecenoic acid	78.46	67.11	66.32	73.15	76.68	72.06	66.54	74.45	78.52	78.67	73.47	73.13
Ricinoleic acid	1.224	4.99	3.11	2.58	2.79	0.91	1.79	4.06	3.35	1.22	3.57	1
Eicosanoic acid	ND	ND	ND	ND	0.43	ND	0.21	0.19	0.81	ND	ND	ND
Nanonedioic acid	ND	1.43	0.09	ND	0.73	1.23	ND	ND	ND	ND	0.16	ND
Tridecanedioic acid	ND	0.13	ND	ND	ND	ND	ND	ND	ND	ND	0.16	ND
Undecylenic acid	ND	ND	ND	ND	ND	ND	0.09	ND	ND	ND	ND	ND
SFA	2.486	4.888	1.79	3.71	3.97	3.14	5.75	3	5.31	2.95	2.08	1.94
USFA	97.364	94.862	97.05	96.14	95.78	96.44	93.32	96.83	94.36	96.89	97.75	97.73
U/S	39.16	19.41	54.21	25..91	24.12	30.71	16.23	32.28	17.77	32.84	47.00	50.37

H= Hass; P= Pinkerton; G=Giant; F=Fuerte; ND = not detected

The amount of oleic acid decreased with decrease in altitude except in giant cultivar where it increased slightly with decrease in altitude (Figure 4.7A). Most previous studies reported oleic acid as the most dominant fatty acid in avocado oils (Dalvao *et al*., 2014: Dreher and Davenport, 2013; Ferreyra *et al.*, 2016) cultivars. The study reported the highest percentage of Palmitoleic acid (C16:1) in Hass grown in zone (3) 27.62%, followed by Giant grown in zone 1 (24.99%) and Fuerte grown in zone 3 (23.60%). There was an increase in amount of palmitoleic acid with decrease in altitude for Hass, Fuerte and Pinkerton. However, for Giant cultivar the trend was reversed (Figure 4.7B).

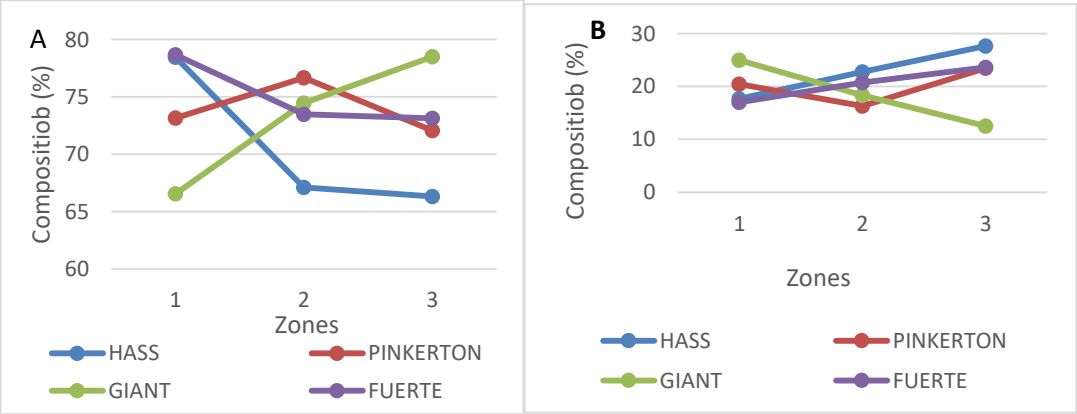


Figure 4.7: Variation of Oleic acid (A) and Palmitoleic acid (B) in different in different avocado cultivars per zone.

The study observed four, seven and five different fatty acids in Hass avocados grown in zone 1, 2 and 3 respectively. Pinkerton avocados grown in zone 1, 2 and 3 had five seven and five different fatty acids respectively. Giant avocados had seven, and six fatty acids in zone 1, 2 and 3 respectively. Fuerte had four, seven and four fatty acids in zone 1, 2 and 3 respectively.

4.4.1 Comparison of Fatty Acids Composition with CODEX Proposed Standards

For edible oils to be deemed pure or of the right grade, they must meet standards, which are a collection of chemical requirements (Hilary *et al*., 2023). The Food and Agriculture body (FAO) and the World Health Organisation (WHO) established CODEX Alimentarius, an international standard development body. In order to develop standards for avocado oil, the CODEX Committee on Fats and Oils has currently formed an Electronic Working Group (EWG) that is co-chaired by the United States of America and directed by Mexico (CODEX, 2021). This is a crucial

step in regulating this product, safeguarding consumer safety, and promoting equitable international commerce and market competition, Hilary *et al*. (2023).

Fatty acid profile, or FAP, is a purity metric used to identify adulterated oils. Avocado oil has been the subject of more research due to the convenience of sample preparation. Through the analysis of samples from a range of harvest times, growing regions, and fruit kinds, this information is helpful in evaluating and considering changes to the present proposed CODEX standards. Additionally, it offers details on the anticipated maximum variance in avocado oil's chemical makeup.

The fatty acid profile of the 12 samples obtained in this study was compared with the current proposed CODEX standards and the results summarized in Table 4.11.

Table 4.11: Fatty acid ranges of tested samples compared to CODEX proposed standards

Fatty Acid	**Range in samples (% of total fatty acids)**	**Proposed CODEX Standards (% of total fatty acids)***	**Number of samples that do not pass current proposed standards (% of total samples)**
C14:0	ND	ND - 0.3	0
C16:0	12.49 - 27.62	11.0 – 26.0	1
C16:1	ND -2.21	4.0–17.1	12
C17:0	ND -0.07	ND - 0.3	0
C17:1	ND	ND - 0.1	0
C18:0	1.5 - 5.36	0.1–1.3	12
C18:1	66.32 -78.46	42.0 –75.0	4
C18:2	ND	7.8–19.0	12
C18:3	ND	0.5–2.1	12
C20:0	ND - 0.81	ND - 0.7	1
C20:1	ND	ND - 0.3	0
C22:0	ND	ND - 0.5	0
C24:0	ND	ND - 0.2	0

Table 4.11 highlights a key conclusion of this investigation, which is that most fatty acids levels are within the ranges of the suggested standards. But in a number of instances, the study's values did not match the standards that are currently being suggested. The fatty acids C14:0, C17:1, C20:1, C22:0 and C24:0 are not always quantified or reported and the ranges proposed for them are ND-0.3, ND-0.1, ND-0.3, ND-0.5 and ND-0.2 respectively, implying all the 12 samples conformed to this standard as they were not detected. C17:0 with a range of ND-0.07 also was within the proposed standard of ND-0.3

One sample for each of the C16:0 and C20:0 standards was beyond the specified range. In comparison to the CODEX recommended standard range of 42.0–75.0%, the samples' oleic fatty acid (C18:1) contents ranged from 66.32 to 78.46%. The suggested standard limits were exceeded by four oleic acid (C18:1) samples. The large range of oleic acid content observed in the samples suggests that the study's factors sufficiently explained a significant portion of the inherent variance in avocado oil. It might be advantageous to slightly increase this limit to prevent samples from failing because of this fatty acid.

The study did not fit into the current proposed standards for C16:1, C18:0, C18:2 and C18:3 where there were 12 of total samples which were outside their respective limits. For palmitoleic (C16:1), the samples were below the 4.0% CODEX limit. All samples had Stearic acid C18:0 ranging from 1.5% to 5.36% which was above the proposed standard range of 0.1% - 1.3%. All the samples had linoleic acid (C18:2) and α-linolenic acid C18:3 were below detectable limit as compared to the lowest proposed standard limit of 7.0% and 0.5% respectively.

Other research (Teng *et al*., 2016; Yanty *et al.*, 2011; Ozdemir & Topuz, 2004; Manaf *et al*., 2018; Henry *et al*., 2023) have also reported variations seen in this study. This is partly because certain places and cultivars with unusual climatic circumstances or cultivars that are not commonly utilised for oil production were analysed. The FAP of avocado oil from five cultivars in Indonesia was measured by Manaf *et al*. (2018). The results showed oleic fatty acid ranges of 21.69 to 55.64%, which was a significant deviation from the study's samples and the suggested criteria for avocado oils. This demonstrates the need of evaluating the significance of some of these natural variables

and examining oils from novel cultivars, geographic locations, etc. to determine if and how standards can accommodate them.

4.5 Elemental Composition of Avocado Cultivars from Murang'a County

Pulp and seed of four avocado cultivars from three ecological zones in Murang'a County were analysed to determine the elemental composition of some essential elements. The results are given in Tables 4.12 and 4.13 for pulp and seed respectively.

Table 4.12: Elemental composition pulp of avocado cultivars from different zones (mg/100g)

	cultivar	K	Na	Ca	Mg	Zn	Fe	Cu
Zone 1	Fuerte	653.7 ^{i}	0.04 ^{e}	27.48 ^{k}	0.14 ^{i}	0.15 ^{d}	0.04 ^{h}	0.0083 ^{i}
	Giant	355.05 ^{a}	0.05 ^{h}	32.20 ^{l}	0.13^{h}	0.14 ^{f}	0.04 ^{j}	0.0080 ^{g}
	Hass	232.8 ^{cd}	0.10 ^{i}	18.01 ^{h}	0.11^{f}	0.15 ^{h}	0.04 ^{i}	0.0086 ^{k}
	Pinkerton	401.7 ^{e}	0.07 ^{i}	1.74^{c}	0.07 ^{a}	0.10 ^{b}	0.01 ^{c}	0.0006 ^{a}
Zone 2	Fuerte	479.1 ^{h}	0.05 ^{g}	27.04^{j}	0.17^{k}	0.16 ^{j}	0.003 ^{a}	0.0083 ^{j}
	Giant	582.1 ^{f}	0.04 ^{d}	1.41 ^{a}	0.15 ^{j}	0.17 ^{k}	0.10 ^{l}	0.0077 ^{f}
	Hass	397.9 ^{e}	0.07 ^{j}	25.86^{i}	0.12 ^{g}	0.21 ^{l}	0.03^{g}	0.0103 ^{l}
	Pinkerton	452.6 ^{c}	0.03 ^{a}	3.47 ^{e}	0.10 ^{e}	0.08 ^{a}	0.03 ^{f}	0.0011 ^{b}
Zone 3	Fuerte	264.7^{cd}	0.05^{f}	4.25^{f}	0.0778 ^{c}	0.13^{d}	0.003^{b}	0.0082 ^{h}
	Giant	442.7 ^{g}	0.07^{k}	1.41^{b}	0.4289 ^{l}	0.14^{g}	0.09^{k}	0.0075 ^{e}
	Hass	169.2 ^{b}	0.03^{c}	3.07^{d}	0.0774 ^{b}	0.11^{c}	0.02^{e}	0.0026 ^{c}
	Pinkerton	424.2 ^{g}	0.03^{b}	4.99^{g}	0.0913 ^{d}	0.14^{e}	0.01^{d}	0.0028 ^{d}

Where: - Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference test.

Table 4.13: Elemental composition seed of avocado cultivars from different zones (mg/100g)

	cultivar	K	Na	Mg	Ca	Fe	Zn	Cu
Zone 1	Hass	721.0^{d}	0.23^{abc}	0.05^{a}	22.8^{d}	0.53^{c}	0.077^{e}	0.0087^{e}
	Fuerte	653.7^{a}	0.17^{ab}	0.08^{b}	31.6^{i}	0.43^{b}	0.049^{a}	0.0079^{a}
	Giant	355.0^{c}	0.16^{a}	0.11^{c}	22^{c}	0.55^{e}	0.065^{c}	0.0086^{e}
	Pinkerton	401.7^{h}	0.19^{ab}	0.13^{f}	16.6^{a}	0.60^{g}	0.088^{f}	0.0082^{b}
Zone 2	Hass	848.6^{e}	0.2^{abc}	0.14^{h}	34.5^{l}	0.68^{i}	0.096^{g}	0.0118^{h}
	Fuerte	479.1^{j}	0.43^{d}	0.18^{k}	30.9^{h}	0.79^{l}	0.152^{i}	0.0165^{k}
	Giant	582.1^{f}	0.4^{d}	0.13^{g}	31.8^{j}	0.78^{j}	0.161^{l}	0.014^{j}
	Pinkerton	452.7^{e}	0.22^{abc}	0.13^{f}	29.5^{f}	0.56^{f}	0.076^{d}	0.0083^{c}
Zone 3	Hass	501.2^{b}	0.17^{ab}	0.11^{d}	17.9^{b}	0.43^{a}	0.062^{b}	0.0084^{d}
	Fuerte	264.7^{g}	0.25^{cd}	0.11^{e}	23.15^{e}	0.62^{h}	0.094^{h}	0.0107^{f}
	Giant	442.8^{k}	0.26^{abcd}	0.14^{i}	30.7^{g}	0.55^{d}	0.159^{k}	0.0138^{i}
	Pinkerton	424.3^{i}	0.21^{abc}	0.15^{j}	31.9^{k}	0.76^{k}	0.152^{j}	0.0109^{g}

Where: - Values are means ± std (n=3); Means followed by different letters are statistically different at $P \leq 0.05$ based on the Tukey's honestly significant difference test.

4.5.1 Potassium

The study reported that avocado contain higher potassium content in Fuerte grown in high altitude (653.7 mg/100g) and the lowest from Fuerte grown in low altitude (169.2 mg/100g). The amount of potassium in Fuerte decreased from 653.7, 479.1 and 264.7 mg/100g from zone 1, 2 and 3 respectively. For Hass, Giant and Pinkerton the highest amount of potassium was in zone 2 and the lowest in zone 3. This indicates that avocado cultivar and geographical growing location have an effect on the amount of potassium in avocado fruit. The analysis indicates significant differences in different cultivar grown in the same geographical area and same cultivar grown in different geographical areas. Healthy adults should aim to consume 3,500–4,700 mg of potassium daily from foods (WHO). To increase this intake, incorporation of a few potassium-rich foods into the diet such as avocado is therefore recommended.

Potassium content in avocado seeds was found to be higher than in the pulp. The highest potassium content was reported in Hass grown in zone 2 (848.6 mg/100g) and the lowest potassium content in Fuerte grown in zone 3 (264.7 mg/100g). Like in the

pulp the potassium content in Fuerte decreased with decrease in altitude while for the rest highest potassium content was reported in zone 2 and the lowest in zone 3.

4.5.2 Sodium

The level of sodium reported in this study ranged from 0.27 mg/100g to 0.96 mg/100g (Figure 12). The highest amount of sodium was reported in Hass in zone 1 (0.96 mg/100g) and the lowest in Pinkerton from zone 2 (0.27 mg/100g). The concentration of sodium in Hass cultivar decreased from 0.96 mg/100g to 0.67 mg/100g and finally to 0.30 mg/100g with decrease in altitude from zone 1 to zone 3 (Figure 4.8). For Giant, Fuerte and Pinkerton the concentration of sodium did not show a linear relationship.

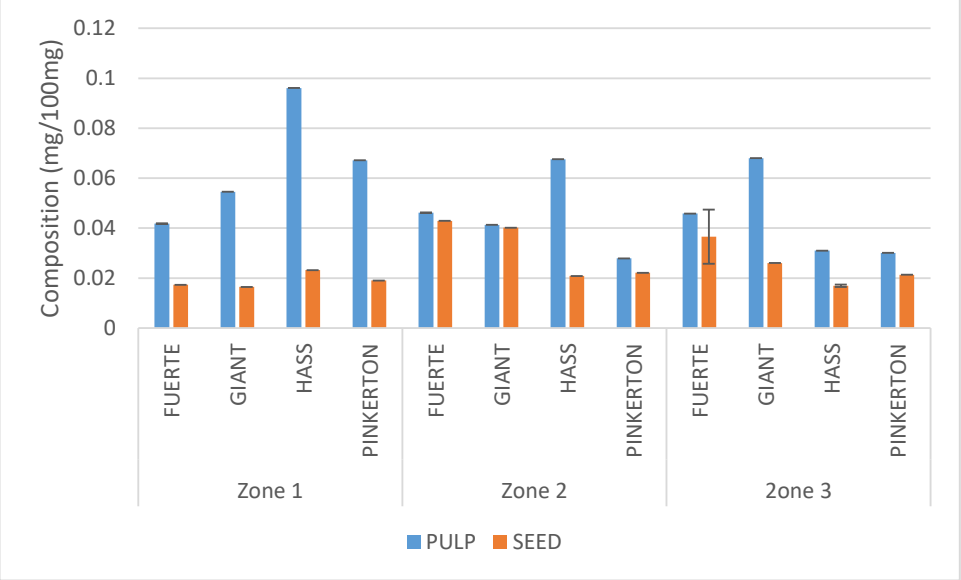


Figure 4.8: Effect of cultivar and fruit part on sodium composition per zone

The concentration of sodium in avocado seeds was lower than in the pulp (Figure 12). The concentration ranged from 0.16 mg/100g to 0.42 mg/100g. The concentration of sodium in Hass avocado seed decreased from 0.23 mg/100g to 0.20 mg/100g and finally to 0.17 mg/100g with decrease in altitude from zone 1 to zone 3. For Giant, Fuerte and Pinkerton the concentration of sodium was highest in zone 2 and lowest in zone 3.

The concentration of sodium in avocado pulp reported in this study is similar to 0.23 mg/100g reported in Nigeria (Maitera *et al*., 2014), and within the range of 0.20 mg/100g-0.80 mg/100g reported by Nair et al., 2018. As for the avocado seed the

concentration sodium was lower than the value of 1.54 mg/100g reported by Isiuku *et al*. (2009).

4.5.3 Calcium

Calcium content was found to be the second highest most abundant mineral in avocado pulp and seed. The concentration of calcium in the pulp ranged from 14.12 mg/100g to 49.95 mg/100g while in the seed it ranged from 16.6 mg/100g to 34.5 mg/100g. The calcium content increased from zone 1 to zone 3 for Hass and Pinkerton. For Giant calcium content was highest in zone 1 and lowest in zone 3 and Fuerte was highest in zone 3 and lowest in zone 2 (Figure 4.9).

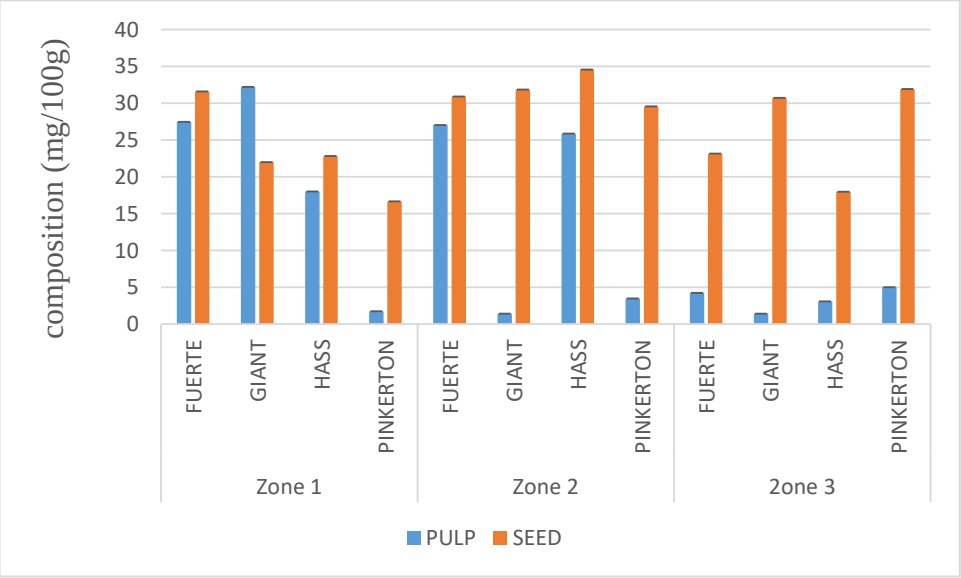


Figure 4.9: Effect of cultivar and fruit part on calcium content per zone

Figure 13 above shows that Calcium content in avocado seeds was highest in Hass avocado seeds grown in zone 2 (34.5 mg/100g) and lowest in Pinkerton grown in zone 1 (16.6 mg/100g). The concentration decreased with decrease in altitude for Pinkerton, increased with decrease in altitude for Fuerte. For Giant and Hass, the concentration was highest in zone 2 and lowest in zone 1.

The value reported are higher than 0.064mg/kg reported by Maitera *et al*. (2014) and 9.0 mg to 9.8 mg/100g those reported by Nair *et al*., 2018, The Dietary allowance for

calcium is 1,300 mg for adults and children aged 4 years and above. Food and Drugs Administration requires food labels to list calcium content. Foods like avocado providing higher (>20%) calcium content and therefore considered good.

4.5.4 Magnesium

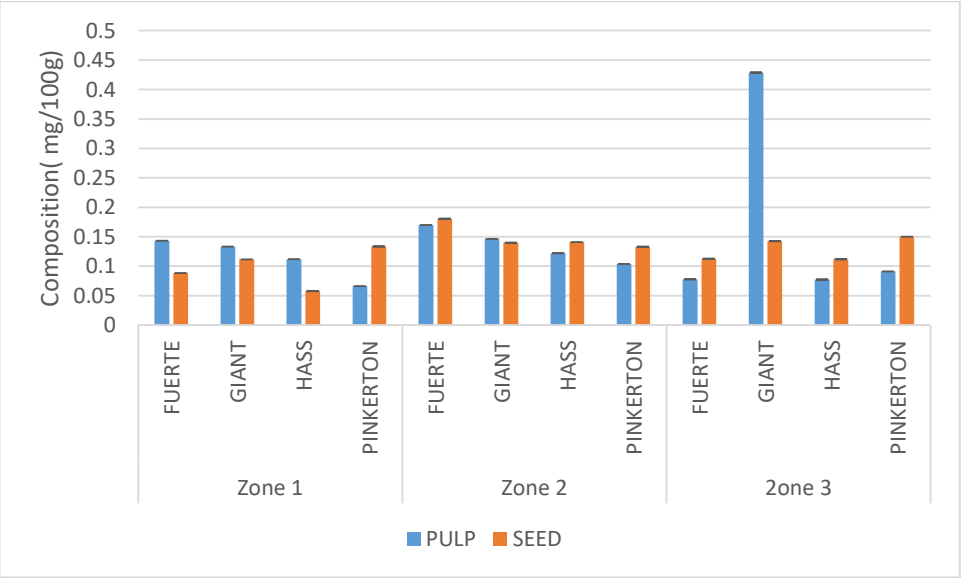


Figure 4.10: Effect of cultivar and fruit part on magnesium composition per zone

The concentration of magnesium reported in this study ranged from 0.07 mg/100g to 0.17 mg/100g for the pulp and 0.05 mg/100g to 0.18 mg/100g for the seed (Figure 4.10). From the study there magnesium content in the seed and the pulp showed very small differences. These values were lower than those reported earlier 1.40 and 1.41 mg/100g for the pulp and seed in RN-7, 1.73 and 1.83 mg/100g for the pulp and seed in RN-8 (Ge *et al.,* 2017). The magnesium content is also similar to 0.15mg/100g for avocado seed reported by Isiuku *et al.* (2009) and 0.103 mg/100g for avocado pulp (Maitera *et al.*, 2014).

4.5.5 Iron

The concentration of iron reported in this study ranged between 0.01 mg/100g to 0.09 mg/100g for the pulp and 0.429 mg/100g to 0.786 mg/100g for the seed. There was significant difference between the iron content in the seed and the pulp (Figure 4.11). The concentration of iron decreased from zone 1 to zone 3 for Hass pulp while it increased for Fuerte pulp from zone 1 to zone 3.

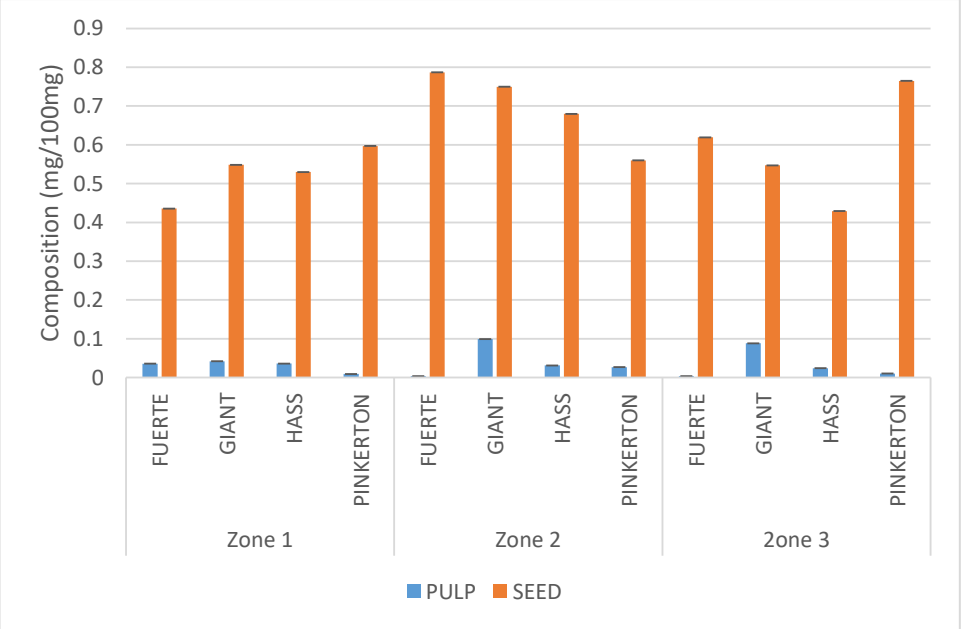


Figure 4.11: Effect of cultivar and fruit part on iron composition per zone

The concentration of iron in avocado seeds was highest in zone 2 for Hass, Fuerte and Giant (0.679 mg/100g, 0.786 mg/100g and 0.749 mg/100g respectively). For Pinerton avocado seed the iron concentration increased from zone to zone 3. The values were lower than those reported in the pulp and the seed of RN-7 and RN-8 (Ge *et al.*, 2017), similar to iron content 0.069 mg/100g reported in the avocado pulp in Nigeria (Maitera *et al.*, 2014) and lower than 0.5, 0.58, 0.6 and 0.4 mg/100g reported in the cultivars of Pollock, Kallar round, Hybrid purple and Fuerte respectively (Nair *et al.*, 2018)

An essential dietary mineral, iron plays a role in many body processes, including the blood's ability to carry oxygen. To stay healthy, the ordinary person needs to absorb only a tiny quantity of iron each day (around 1.5 mg for menstrual women and 1 mg for adult males). However, we must ingest many times that quantity in order to do this. This is because only a portion of the iron in the food we eat is absorbed by our bodies.

4.5.6 Zinc

The concentration of zinc in the pulp ranged from 0.078 mg/100g to 0.2 mg/100g while in the seed the range was 0.049 mg/100g to 1.59 mg/100g. For zone 1 the zinc content in avocado pulp was higher than in the seed. The concentration of zinc was

highest in zone 2 and lowest in zone 1 for Hass, Fuerte and giant cultivar. For Pinkerton zinc content was highest in zone 3 and lowest in zone 2 (Figure 4.12).

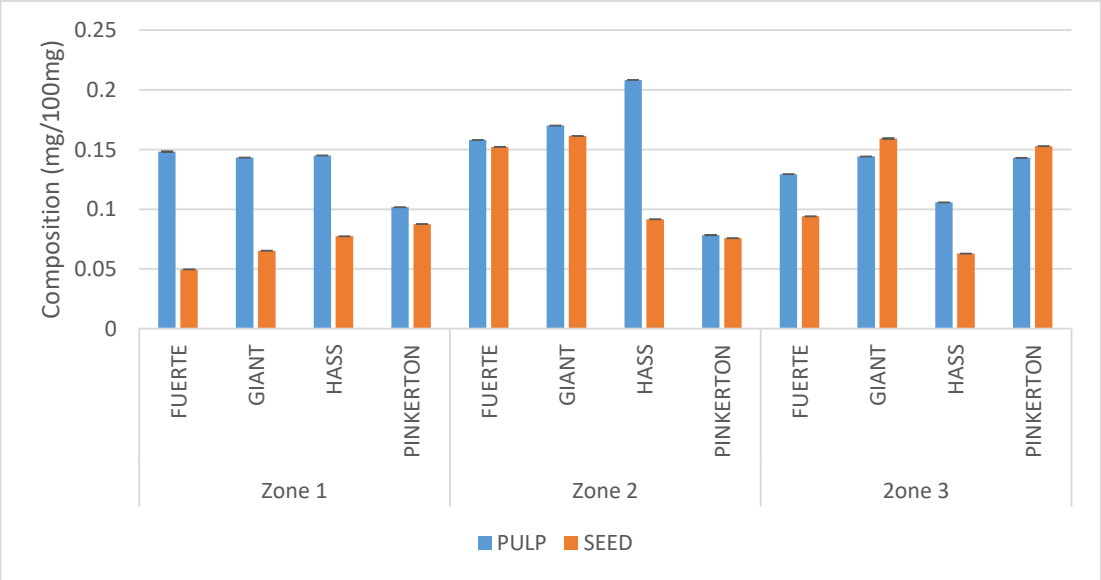


Figure 4.12: Effect of cultivar and fruit part on zinc composition per zone

In the seed the zinc content was highest in zone 3(1.52 mg/100g) and lowest in zone 2 (0.0758 mg/100g). For Giant, Hass and Fuerte zone 2 had the highest zinc content with zone 3 having the lowest. The results comparable with 0.07 mg/100g and 0.10 mg/100g obtained from RN-7 and RN-8 avocado seeds (Ge *et al*., 2017) but lower than 0.18 mg/100mg obtained in avocado seed by Isiuku *et al*., 2009. The Recommended Dietary Allowance (RDA) for adults 19+ years is 11 mg a day for men and 8 mg for women.

4.5.7 Copper

Copper is a trace element required in the human body. The study reported copper concentration in the range of between 0.0079 mg/100g and 0.0165 mg/100g in the seeds and 0.001 mg/100g to 0.0086 mg/100g in the pulp (Figure 4.13). Fuerte and Giant had relatively the highest concentration of copper in all zones that showed significant differences. Pinkerton had the lowest concentration of 0.001, 0.0011and 0.002 mg/100g in zone 1, 2 and 3 respectively. Hass pulp had 0.086 mg, 0.01 mg and 0.002 mg/100g in zone 1, 2 and 3 respectively. These values are lower than 0.47 mg/100g reported in Ethiopia (Tegegne *et al*., 2017).

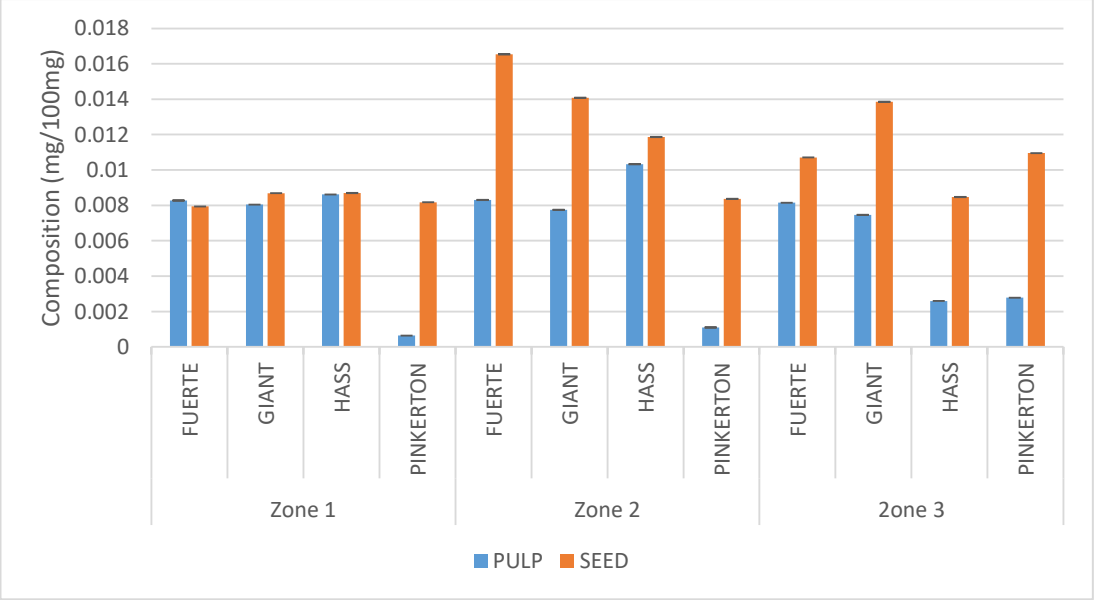


Figure 4.13: Effect of cultivar and fruit part on copper composition per zone

The copper content of avocado seeds ranged from 0.007 mg/100g to 0.165 mg/100g. The content of copper in Zone 1 was approximately 0.008 mg/100g for all cultivars. Zone 2 had the largest concentration among the zones, whereas Zone 1 had the lowest. The average daily consumption of copper from food for adults aged 20 and above is 1,100 mcg for women and 1,400 mcg for men. Children should get between 900 and 1,100 mcg per day from foods and supplements, while people aged 20 and older should consume 1,400 to 1,700 mcg per day.

Table 4.14 and 4.15 below shows the two-way ANOVA elemental analysis for the pulp and seed respectively. From the analysis, there was significant difference in mineral elements content.

Table 4.14: Two-way ANOVA analysis for elemental analysis in pulp

Parameter	***Source of Variation***	***SS***	***df***	***MS***	***F***	***P-value***
K	Zone	34.16111	2	17.08056	46.84132	5.18E-09
	Cultivar	2449.999	3	816.6664	2239.607	1.66E-29
Na	Zone	0.003274	2	0.001637	1800877	7.66E-63
	Cultivar	0.002983	3	0.000994	1093926	9.43E-62
Mg	Zone	0.018547	2	0.009274	203564.7	1.76E-51
	Cultivar	0.121181	3	0.040394	886690.9	1.17E-60
Ca	Zone	1681.38	2	840.6898	2.1E+10	1.2E-111
	Cultivar	1292.583	3	430.861	1.08E+10	1.1E-109
Fe	Zone	0.00068	2	0.00034	1224500	7.85E-61
	Cultivar	0.022949	3	0.00765	27538267	1.46E-78
Zn	Zone	0.003661	2	0.00183	20590508	1.54E-61
	Cultivar	0.012576	3	0.004192	47158838	2.29E-81
Cu	Zone	1.66E-05	2	8.31E-06	124660.6	6.32E-49
	Cultivar	0.000266	3	8.87E-05	1330835	8.97E-63

Table 4.15: Two-way ANOVA analysis for elemental analysis in seed

Parameter	***Source of Variation***	***SS***	***df***	***MS***	***F***	***P-value***
K	Zone	1907.772	2	953.8862	7631.09	2.24E-34
	Cultivar	2084.789	3	694.9297	5559.438	3.12E-34
Na	Zone	0.000939	2	0.000469	16.03782	3.78E-05
	Cultivar	0.000887	3	0.000296	10.1001	0.000172
Mg	Zone	0.015741	2	0.00787	88542.99	3.83E-33
	Cultivar	0.006293	3	0.002098	23597.56	9.25E-42
Ca	Zone	448.2378	2	224.1189	3.2E+09	7.7E-102
	Cultivar	74.73659	3	24.9122	3.56E+08	6.71E-92
Fe	Zone	0.169411	2	0.084705	2.75E+31	0
	Cultivar	0.043877	3	0.014626	4.75E+30	0
Zn	Zone	0.019119	2	0.00956	22641275	4.91E-62
	Cultivar	0.012096	3	0.004032	9549858	4.81E-73
Cu	Zone	0.000114	2	5.72E-05	643190.9	1.78E-57
	Cultivar	6.07E-05	3	2.02E-05	227443.8	1.44E-53

4.6 Summary

This chapter presents the findings of the thesis research, focusing on a detailed analysis of different avocado cultivars from Murang'a County. The subtopics indicate a systematic presentation of the results. The first major section, Proximate Composition, details the fundamental chemical makeup of the avocados, reporting specific data on moisture, ash, and lipid content. This establishes the basic physical and chemical properties of the samples studied.

Following this, the chapter shifts to the Physicochemical Properties of the Extracted Oils. This section likely presents data on various key metrics used to determine the quality and stability of the avocado oil, such as specific gravity, acid value, iodine value, saponification value, and peroxide value. These results are crucial for understanding the oil's potential applications and shelf life. The final major section focuses on the Elemental Composition of the Avocado Cultivars. This is a detailed report on the mineral content of the fruit, providing specific values for a range of elements. The subtopics list key minerals and trace elements, including Potassium, Sodium, Calcium, Magnesium, Iron, Zinc, and Copper, which contributes to a comprehensive understanding of the nutritional profile of the avocados.

Overall, this chapter moves from a foundational analysis of the fruit's composition to a specific characterization of its oil and a detailed inventory of its mineral content, all of which likely contributes to the conclusions of the thesis.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The parameters that were investigated include; oil content with a range between 59.44% -75.32% on dry weight basis. Fuerte was found to have the highest mean yield followed by Hass then Giant and Pinkerton the lowest. Avocados grown in zone 3 had the lowest oil yield. The moisture content ranged between 59.30% for Fuerte grown in zone 3 to 84.31% for Pinkerton grown in zone 1. Ash content indicated that Giant cultivar had a higher mineral content in all zones with a mean of 1.53% followed by Pinkerton with a mean of 1.32% while Hass had the least ash content with a mean of 0.96%. The study reported that the dietary fibre was highest in zone 1 for Hass (3.16%), Pinkerton (6.43%) and Giant (4.33%) followed by zone 3 and lowest in zone 2. For Fuerte the dietary fibre was lowest in zone 1 (1.83%). The dietary fibre increased with decrease in altitude. The proximate parameters investigated varied significantly and therefore the null hypothesis as rejected.

The physicochemical properties of oil investigated are; specific gravity ranged from 0.85 to 0.92. This indicates that avocado oil is less dense than water. Hass, Pinkerton and Giant cultivars grown in higher altitude area with relatively cold weather conditions had a higher specific gravity (>0.9) suggesting higher unsaturated oil. The acid values ranged from 0.18 to 0.98. Fuerte cultivar had the highest acid value in all the zones while Hass has the lowest. A saponification value range of 123.42 to 187. On average avocado fruits collected in zone 1 had a higher saponification value (151.00) while zone 2 had the lowest saponification value (144.92). Iodine value of the oils ranged from 54 to 107. Peroxide values ranged from 0.9 to 2.3. The physicochemical properties investigated varied significantly among the zones and cultivars, therefore the null hypothesis was rejected.

Four fatty acids were present in all cultivars while seven fatty acids were present in one or more cultivars. The most dominant fatty acid was oleic acid followed by palmitoleic acid. The amount of oleic acid decreased with decrease in altitude except in giant cultivar where it increased slightly with decrease in altitude. There was an increase in amount of palmitoleic acid with decrease in altitude for Hass, Fuerte and Pinkerton, however for Giant cultivar the trend was reversed.

The elemental concentration of potassium, magnesium, calcium, iron and copper was higher in the seed than in the pulp while the concentration of sodium and zinc was higher in the pulp compared to the seed. There were significant differences in elemental composition between the zones and the cultivars. The null hypothesis was therefore rejected.

5.2 Recommendations

5.2.1 Recommendations for nutrition applications

i. Avocado pulp contains high quality lipids and essential minerals. Dry powdered avocado pulp should be used in supplementation of various food products.
ii. Data obtained from this study should be used as basis for conforming to nutritional labelling requirements for food and agricultural products.
iii. Avocado seed which contributes to environmental pollution during avocado oil processing contains high content of minerals. Upon evaluation of the seed's bioactive components, it should be used as animal feed supplement.

5.2.2 Recommendations for policy

To maximize avocado returns for growers, fruit needs to be supplied to the consumer with both internal and external quality acceptable to the consumer. National guidelines should therefore be developed to enhance avocado quality in Kenya. Such guidelines include;

i. Developing nutritional labelling laws and ensuring that avocado fruits meet the minimum requirements for export market.
ii. Developing and maintaining export documentation with regard to different avocados cultivars as well as geographical growing areas.

5.2.3 Recommendations for research

i. The study investigated four avocado cultivars grown in Murang'a County. However avocados are grown in different parts of the country and therefore a similar study should be done to compare the avocados grown in Murang'a County to others areas.
ii. The study also investigated four cultivar whereas they are many cultivars grown in Murang'a county. It is necessary to conduct studies on the other

cultivars to obtain a comprehensive comparison on the parameters investigated.

iii. All the fruits investigated in this study were collected during the same season. Composition of avocado has been found to vary from season to season, to characterize Murang'a County avocados comprehensively, seasonal variation in composition should be studied.

iv. The composition and the quantity of different Fatty acids in avocados vary with maturity. Further study should be done to investigate the variation of the various fatty acids with maturity.

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APPENDICIES

Appendix 1: Avocado Fruits



Hass Avocado



Giant Avocado



Fuerte Avocado



Pinkerton Avocado

Appendix 2: Publications

This thesis has produced the following publications:

Journal papers published:

Wanjiru, S. N., Opiyo, S. A., Njoroge, P. W., & Mugendi, B. J. (2025). Elemental Composition of Pulp and Seed of Avocado Cultivars from Murang'a County.

Wanjiru, S. N., Opiyo, S. A., Njoroge, P. W., & Mugendi, B. (2025). Effect of Cultivar and Altitude on Fatty Acid Composition of Avocado from Murang'a County.

Wanjiru, S. N., Opiyo, S. A., Njoroge, P. W., & Mugendi, B. (2025). Proximate analysis and physicochemical properties of oil from four avocado cultivars from Murang'a County, Kenya. American Journal of Chemistry, 15(2), 50–58. https://doi.org/10.5923/j.chemistry.20251502.03

Conferences Attended:

Wanjiru, S. N., Opiyo, S. A., Njoroge, P. W., & Mugendi, B. (2023, Nov 9). Evaluation of elemental composition of pulp and seed from four different avocado varieties grown in Murang'a County, Kenya. [Blended platform]. Murang'a University of Technology 2nd international conference, Murang'a, Kenya.

Wanjiru, S. N., Opiyo, S. A., Njoroge, P. W., & Mugendi, B. (2023, Nov 9). A review of fatty acid components in avocado. [Blended platform]. Murang'a University of Technology 2nd international conference, Murang'a, Kenya.