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Effect Of Cultivar And Altitude On Fatty Acid Composition Of Avocado From Murang'a County

Samuel N. Wanjiru, Sylvia A. Opiyo, Peter W. Njoroge, Beatrice Mugendi
Department Of Physical And Biological Sciences, Murang'a University Of Technology, Kenya

Abstract
Fatty acids are essential for the structure and function of biological systems, and they are the primary source of energy. Avocado oil has high levels of omega-6 and omega-9 fatty acids, as well as natural antioxidants, which help lower total cholesterol, triacylglycerol, and low-density lipoprotein (LDL) cholesterol while maintaining healthy high-density lipoprotein (HDL) cholesterol levels. Avocado's fatty acid profile varies depending on cultivar, plant portion, ripening stage, geographical location, and sampling procedures. The purpose of this study was to examine the effect of cultivar and altitude on the fatty acid composition of avocado grown in Murang'a County, Kenya. Four different avocado cultivars were sampled from Murang'a County's three climate zones: Zone 1 (above 2,200m a.s.l.), Zone 2 (1,700m-2,100m a.s.l.), and Zone 3 (below 1,600m a.s.l). The oil was extracted from dry powdered pulp using Soxhlet extraction with petroleum ether 40-60. The fatty acid profile was evaluated using GC-MS. Oil output dropped as altitude decreased from Zone 1 to Zone 3. A total of 11 different fatty acids were identified, namely 9-hexadecenoic (palmitoleic), hexadecanoic (palmitic), 9-octadecenoic (oleic), stearic, 12-hydroxy oleic acid (ricinoleic), nonanedioic (azelaic), tridecanedioic (brassylic), eicosanoic (arachidic), undecylenic, heptadecanoic (margaric), and dodecanoic (lauric). All cultivars had four fatty acids: 9-octadecenoic, octadecanoic, 12-hydroxy oleic, and 9-hexadecenoic, with the most prevalent being 9-octadecenoic (oleic). Except for the Giant cultivar, the amount of oleic acid reduced as altitude fell. The fatty acid makeup was comparable to that of other avocados published before.

Keywords: *Avocado; Fatty acid; Cultivars, Altitude*

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I. Introduction

Avocado is among the most economically significant fruit crops globally. In recent years, it has gained considerable attention as a natural functional food due to its exceptional nutritional value and health benefits [1, 2]. Rich in unsaturated fatty acids, fat-soluble vitamins like vitamin E and B6, β-carotene, sterols, fiber, protein, magnesium and potassium, avocado is highly nutritious [3, 4]. Its oil contains notable amounts of omega-6 and omega-9 fatty acids, along with natural antioxidants that help lower total cholesterol, triacylglycerol, and low-density lipoprotein (LDL)-cholesterol while maintaining healthy high-density lipoprotein (HDL)-cholesterol levels [5]. The fruit's high digestible oil content and low sugar levels make it an excellent energy source and a valuable addition to diabetic diets. Consuming avocado has been linked to preventing and managing diet-related conditions such as cardiovascular disease, diabetes, obesity, hypertension, chronic inflammation, dyslipidemia, and even cancer [5]. Additionally, its high unsaturated fat content enhances skin permeability and offers sun protection. Avocado extracts have also demonstrated antibacterial, antioxidant, and antiviral properties [6].

Fatty acids are necessary for both the structure and operation of biological systems, and they constitute a primary source of energy. They have an important role in mammalian cells, maintaining membrane integrity and acting as signaling molecules [7]. While the human body can create numerous fatty acids, it is unable to produce important polyunsaturated fatty acids (PUFAs) such as linoleic acid (LA) and alpha-linolenic acid (ALA), which must be received through diet [8]. ALA is predominantly contained in vegetable oils, almonds, flax seeds, flaxseed oil, leafy greens, and certain animal fats, particularly those derived from grass-fed livestock. Short-chain polyunsaturated fatty acids are transformed to long-chain fatty acids after consumption. Linoleic acid is the precursor for additional omega-6 fatty acids, whereas ALA is the precursor for omega-3 fatty acids [9]. The major omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are mostly derived from fish and are commonly referred to as marine omega-3s.

Previous studies have shown that avocado contains saturated, monosaturated as well as polysaturated fatty acids. The saturated fatty acids include myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), behenic (22:0) and tetraeicosanoic acid (24:0); the monosaturated fatty acids include myristoleic (C14:1), palmitoleic (C16:1), oleic (C18:1), eicosenoic (C20:1) and docosenoic (22:1); while the polyunsaturated ones include linoleic (18:2), linolenic (18:3n-3), linolenic (18:3n-6), ecosadienoic (C20:2) and docosahexaenoic acid (22:6n-3). Some of fatty acids demonstrated antimicrobial, antioxidant and anti-inflammatory effects among others [3]. The fatty acids profile in avocado vary with cultivar, plant part, stage of ripening, geographical location and sample processing methods [10, 11].

When fatty acid composition of three Indonesian cultivars namely Merah bundar, Ijo bundar and Ijo panjang together with imported Fuerte and Shepard avocado were determined, all the avocado oils contained palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids [12]. Merah bundar cultivar contained oleic (43.44%), palmitic (28.45%), linoleic (16.27%), and palmitoleic (9.81%). The Fuerte avocado oil contained oleic acid (55.64%), palmitic (22.13%), linoleic (14.18%) and palmitoleic (6.11%) [12]. In a study of four avocado fruit varieties (Ettinger, Fuerte, Hass and Reed) from Morocco, the most predominant fatty acids were oleic acid palmitic and linoleic acid (in that order) with different proportions depending on the variety. The Reed variety reported the highest amount of oleic acid (61.18%), followed by Fuerte 57.5% while the Hass variety recorded the lowest amount (54.53%). Ettinger had the highest proportion of unsaturated fatty acids with a value of 84.04% [13].

Fatty acid profile and composition of the Hass avocado was found to vary with region of cultivation [14]. Orchards between 1,340 and 2,420 m a.s.l. were selected and the fatty acid profile and content of the fruits were analyzed. Oleic acid showed the highest percentage for all of the locations and its percentage decreased drastically at lower altitudes, meanwhile the percentage of palmitoleic and linoleic acids increased in these orchards. The oleic/palmitoleic, linoleic/ palmitoleic, and oleic/linoleic indexes increased significantly at higher altitudes [15]**.** In analysis of fatty acid profile of Hass avocado from different altitudes in Tenerife, avocados from orchards at middle altitudes in the northern area and had higher fat and dry matter contents than orchards located at low altitudes, while the opposite occurred in the southern area [16]. The influence of the production altitude on the percentage of oleic acid was different according to the area. [16].

Humans have always relied on plants to meet their basic needs, including food and medicine [17-26]. Plants provide a genuine alternative to primary healthcare services in Sub-Saharan Africa's developing countries [27-32]. Previous research has shown that plant extracts can help manage diseases and pests. Plants are known to produce essential secondary metabolites that are poisonous to pathogenic diseases [33-41]. The hunt for natural replacements for synthetic chemicals often used in the food, pharmaceutical, and cosmetic industries has gained traction in recent years [42-45]. Plant extracts are recommended for disease control since they are nontoxic to non-targeted organisms and environmentally friendly [46-56]. Furthermore, it is exceedingly unlikely that hazardous microorganisms will develop resistance to herbal medications.

This study investigated the fatty acid profiles of four cultivars of avocado grown in different agro ecological zones in Murang'a County in Kenya.

II. Materials And Methods

Murang'a County covers 2,558.8 km² and is located between latitudes 0° 34' and 10° 7' South and longitudes 36° and 37° 27' East. The elevation ranges from 914 to 3,353m above sea level. It has three climate zones: western, subtropical, and arid/semi-arid. The county's fields are spread over several altitude zones. The top zone (above 2,200m) is located in Kanderendu, Kigumo constituency. The intermediate zone (1,700m-2,100m) includes the Gatanga and Kihumbu-ini wards in the Gatanga constituency, while the lower zone (below 1,600m) includes the Ichagaki and Kamahuha regions of the Maragua Constituency. These changes in altitude and climate have an impact on agricultural activity and crop output throughout the region.

Four different cultivars of avocado samples were taken from Murang'a County's three climate zones (high, medium, and lower). The zones were numbered as follows: Zone 1 (high altitude areas), Zone 2 (medium altitude areas), and Zone 3 (low altitude areas). The avocados gathered were randomly assigned numbers based on cultivar and zone of collection. The samples were separated and stored for 4-7 days to ripen. The ripe fruits were extensively cleansed with water to remove soil and other debris from their surfaces. The ripe fruits were weighed and then split longitudinally to remove the seeds. Thereafter, the peel was removed and the pulp was cut into small pieces, and crushed in a mortar. The oil was extracted using Soxhlet extraction method and using petroleum ether 40-60 as the solvent [12]. A homogeneous sample of avocado pulp was oven-dried for 24 hours at 70°C before being milled into fine powder. 10g of dried sample was mixed with petroleum ether and extracted in a Soxhlet extractor for 8 hours at 60°C. The resulting extract was evaporated in a rotary evaporator at 95°C to remove the solvent. The oil was weighed and used for fatty acid analysis.

The method described by Ichihara & Fukubayashi [57] for converting oil into fatty acid methyl esters was used. 0.2g of oil was heated in a screw-tight test tube at 85°C for 2 hours with 8mL of 95% methanolic hydrochloric acid added. After cooling to room temperature, the mixture was put into a separating funnel, 4mL of n-hexane was added, and shaken vigorously for 30 seconds. The hexane layer was obtained after the mixture had been allowed to stand. To eliminate any acid, the extract was washed with four portions of water. The mixture was separated and dried with anhydrous sodium sulphate.

GC-MS (Agilent Technologies, Inc., 8860 GC system, CA, USA) was utilized for fatty acid analysis. Chromatographic separation of FAMEs was performed through an HP-5MS UI column (100 m ×0.250 mm × 0.25 mm, Agilent Technologies, Inc., CA, USA). Helium (>99.999% purity) at a constant flow rate of 1.0mLmin^{-1} was used as the carrier gas. The injector temperature was set to 240°C and the injection volume was set to 1.0 µL. All GC injections were introduced to the instrument using the split mode with a 1:10 split ratio. The initial column temperature was set at 125°C and held for 1.5 min, followed by an increase in temperature at a rate of 10°C min^{-1} to 180°C, then the temperature was held for 2min, followed by an increase in temperature at a rate of 3°C min^{-1} to 200°C, then the temperature was held for 20 min followed by an increase in temperature at a rate of 5°C min^{-1} to 240 °C, then the temperature was held for 10 min.. The temperatures for the mass transfer line and ion source were set to 240°C and 230°C, respectively. The quadrupole temperature was set at 150°C. FAME samples were ionized in the electron impact ionization (EI) in positive mode at 70 eV. 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. The fatty acids were identified by comparison of the spectra data obtained with those available in the Wiley and NIST libraries (Wiley Registry TM, 8th Edition Mass Spectral Library and the NIST Mass Spectral Library [58].

III. Results And Discussion

The oil content of sampled avocado cultivars ranged between 59.44%-76.06% on dry weight basis (Figure 1). 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 highest mean percentage oil yield was found 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 71.66% followed by zone 1 at 71.12% and the lowest oil content in zone 3 at 65.27%. This shows that cultivar has an influence on the oil content of the avocado fruits. There was significant difference on the oil yield between the cultivars and between the geographical growing environments at $P \leq 0.05$.

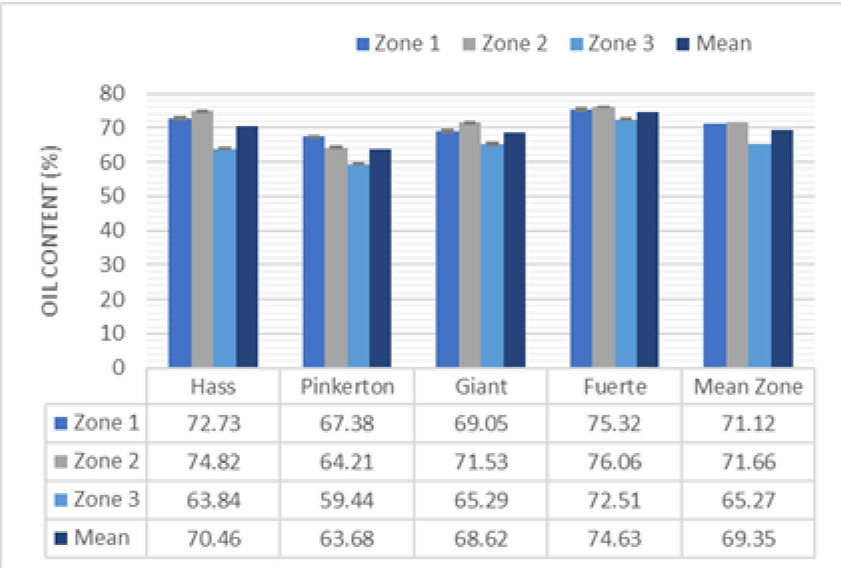

Figure 1: Variation of oil yield per cultivar per zone

The percentage oil yields reported in this study are higher than 36.18%-47.52%, 36.4% and 18.9% earlier reported in Indonesia, Nigeria and Colombia respectively [12, 15, 59]. The oil yield was lower in all cultivars in grown in Zone 3 (low altitudes). Similar observation was obtained by Carvalho *et al*. [15] where Hass avocado was harvested from orchards at different altitudes. At 2420m asl, 1900m a.s.l. and 1510m a.s.l., Hass avocado yielded 18.9, 14.1 and 7.8% oil content respectively [15]. 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.

A total of 11 different fatty acids were identified namely 9-hexadecenoic (palmitoleic), hexadecanoic (palmitic), 9-octadecenoic (oleic), octadecanoic (stearic), 12-hydroxy oleic acid (ricinoleic), nonanedioic (azelaic), tridecanedioic (brassylic), eicosanoic (arachidic), undecylenic, heptadecanoic (margaric) and dodecanoic (lauric) acid (Figures 2- 4). The mass spectrum of 9-octadecenoic (oleic) acid methyl ester (Figure 2) 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 [60]. The mass spectrum of 9-octadecanoic (stearic) acid methyl ester (Figure 3) 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 [60] (Abel et al., 2019). The mass spectrum of hexadecanoic (palmitic) acid methyl ester (Figure 4) 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 [60].

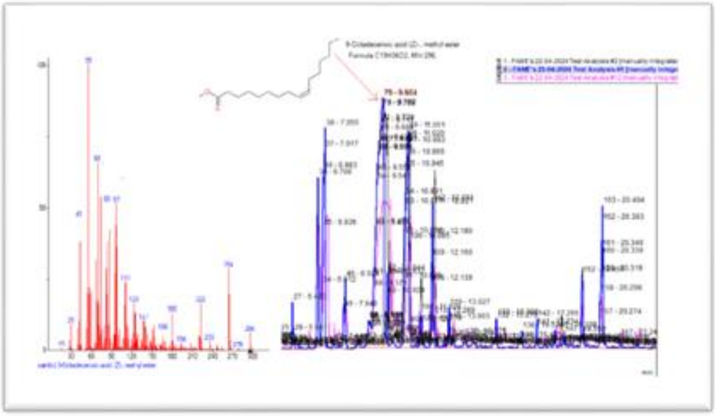

Figure 2: Mass spectrum and GC chromatogram for 9-octadecanoic acid methyl ester

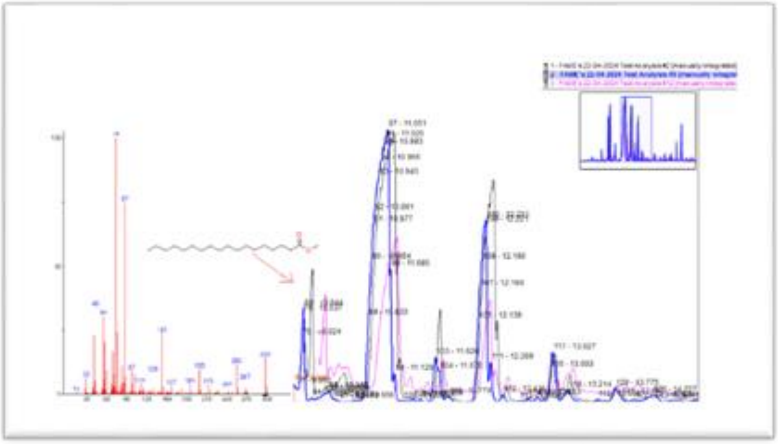

Figure 3: Mass spectrum and GC chromatogram for methyl stearate

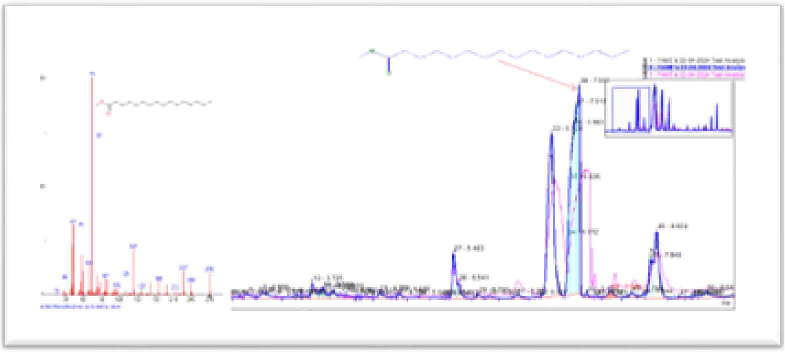

Figure 4: Mass spectrum and GC chromatogram for hexadecanoic methyl ester

Four fatty acids were present in all cultivars while seven fatty acids were present in one or more cultivars from different zones (Table 1 & Figure 5). The most dominant fatty acid was oleic acid whose concentration ranged from 66.32% to 78.67% and as found in all cultivars from the three zones. The second most dominant fatty acid was palmitoleic acid whose concentration ranged from 12.49% to 27.62%. Fuerte grown in zone 1 had the highest percentage of oleic acid 78.67% followed by Giant grown in zone 3 (78.52%). Hass grown in zone 1 had 78.46% while Pinkerton had 73.15%. The amount of oleic acid decreased with decrease in altitude except in giant cultivar where it increased slightly with decrease in altitude. Previous studies reported oleic acid to be the most dominant fatty acid in avocado oils [61, 62]. The highest percentage of palmitoleic acid (27.62%) was recorded in Hass grown in zone 3, 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.

Table 1: Fatty acids composition of avocado cultivars from different zone in Murang'a County

Fatty acid		Fatty acid composition (%)											
	RT	H 1	H 2	H 3	P1	P2	P3	G1	G2	G3	F1	F2	F3
Nonanedioic	3.02	ND	1.43	0.09	ND	0.73	1.23	ND	ND	ND	ND	0.16	ND
Tridecanedioic	4.13	ND	0.13	ND	ND	ND	ND	ND	ND	ND	ND	0.16	ND
10-Undecylenic	4.50	ND	ND	ND	ND	ND	ND	0.09	ND	ND	ND	ND	ND
9-Hexadecenoic	6.92	17.68	22.76	27.62	20.41	16.31	23.47	24.99	18.32	12.49	17	20.71	23.6
Dodecanoic	7.00	ND	ND	ND	ND	ND	ND	0.04	ND	ND	ND	ND	ND
Heptadecanoic	8.30	ND	ND	ND	ND	ND	ND	0.05	0.04	0.07	ND	ND	ND
9-Octadecenoic	9.40	78.46	67.11	66.32	73.15	76.68	72.06	66.54	74.45	78.52	78.67	73.47	73.13
Octadecanoic	10.05	2.49	2.54	1.7	1.5	2.4	1.91	5.36	2.43	3.4	2.95	1.55	1.94
12-hydroxy oleic	13.23	1.22	4.99	3.11	2.58	2.79	0.91	1.79	4.06	3.35	1.22	3.57	1.00
Eicosanoic	13.80	ND	ND	ND	ND	0.43	ND	0.21	0.19	0.81	ND	ND	ND
Hexadecanoic	18.35	ND	0.79	ND	2.21	0.43	ND	ND	0.34	1.03	ND	0.21	ND

H1 = Hass from zone 1; H2 = Hass from zone 2; H3 = Hass from zone 3; P1 = Pinkerton from zone 1; P2 = Pinkerton from zone 2; P3 = Pinkerton from zone 3; G1 = Giant from zone 1; G2 = Giant from zone 2; G3 = Giant from zone 3; F1 = Fuerte from zone 1; F2 = Fuerte from zone 2; F3 = Fuerte from zone 3; ND = not detected

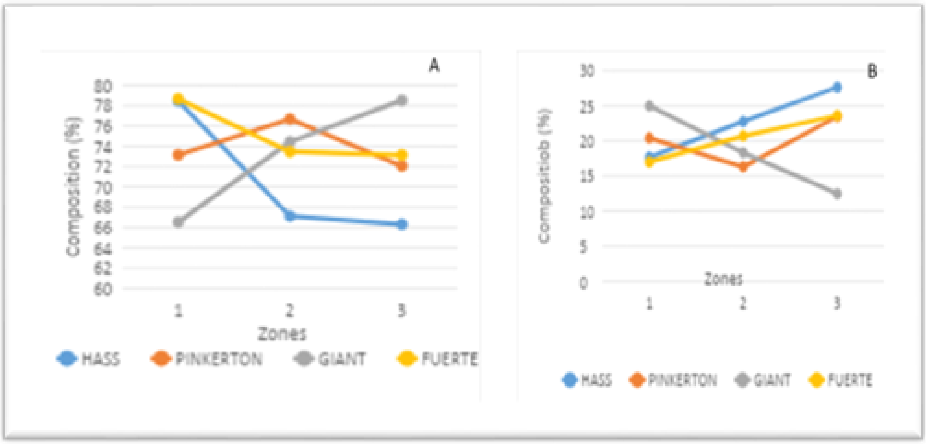

Figure 5: Variation of oleic acid (A) and palmitoleic acid (B) in different avocado cultivars per zone

Four, seven and five different fatty acids were identified 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 eight, seven, and seven fatty acids in zone 1, 2 and 3 respectively. Fuerte had four, seven and four fatty acids in zone 1, 2 and 3 respectively. Some unusual fatty acid including nonanedioic, heptadecanoic, ricinoleic and undecylenic acid were also reported from the oil samples. Nonanedioic (azelaic) was previously reported from mesocarp of avocado [63]. Evaluation of the volatile compounds of Fuerte avocado seed oil found heptadecanoic acid to be the major components at a concentration of 32.5% [64]. Heptadecanoic was also reported in small quantities in avocado oils and avocado seed [65, 66]. Ricinoleic acid has been reported in may plant extracts including castor beans, sclerotium of ergot (*Claviceps purpurea*), olive oil, cottonseed oil, and grape seed oil [67]. Ricinoleic acid has many applications including used in cosmetics, pharmaceuticals, and lubricants industries [67]. I also has anti-inflammatory and analgesic, laxative and labor induction effects [67]. It is currently being explored as a petrochemical substitute in various industrial processes [68, 69]. Undecylenic acid which has antifungal properties and is used to treat skin fungal infections was previously reported in avocado oil and seeds [70].

There was a huge difference in the composition of unsaturated fatty acids and saturated fatty acids present in the avocado samples with the unsaturated fatty acids dominating (Figure 6). Unsaturated fatty acids composition ranged from 93 to 98% an all the samples while the saturated ones ranged from 2 to 6%. Unsaturated fatty acids are considered "good" fats because they help to lower LDL (bad) cholesterol, improve heart health, and reduce the risk of heart disease and stroke, while also providing essential nutrients for cell function and development [71, 72]. Previous studies have shown that avocados are a very good source of healthy unsaturated fatty acids, particularly monounsaturated fats, which are known for their heart-healthy benefits [3].

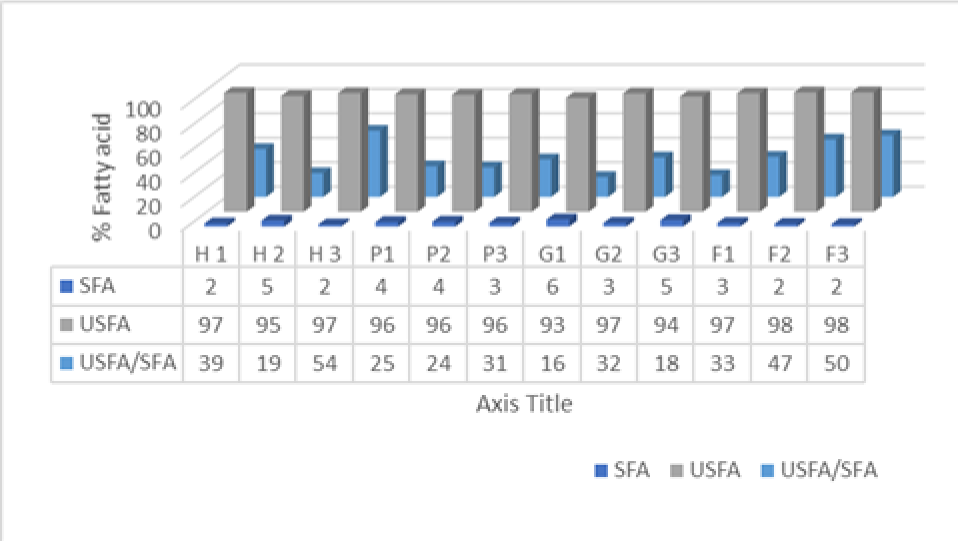

Figure 6: Saturated fatty acids and unsaturated fatty acids composition

IV. Conclusion

Avocado grown in Murang'a County contain a wide range of fatty acids namely palmitoleic, palmitic, oleic, stearic, ricinoleic, azelaic, brassylic, arachidic, undecylenic, margaric and lauric acid. The fatty acid profiles vary with the cultivar and zone of production and are comparable to avocados from other regions.

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