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Proximate Analysis and Physicochemical Properties of Oil from Four Avocado Cultivars from Murang'a County, Kenya

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Abstract Avocado has a high nutritional value with a high content of unsaturated fatty acids, vitamins, phytochemicals, fiber, protein, and minerals such as magnesium and potassium. However, it is difficult to determine the harvesting stage especially for green-skinned varieties. Maturity of avocado is determined by analysis of parameters such as maturity index, oil content and minerals content. However, several factors affect chemical the profile including seasonal variation, cultivar and geographical growing area. The objective of this study was to determine the proximate and physicochemical properties of avocado from Murang'a County, Kenya. Samples of four different cultivars were collected from three geographical zones. Proximate analysis was done on fresh avocado pulp. Oil was extracted from avocado pulp using petroleum ether. The proximate composition of avocado of the four cultivars ranged as follows: moisture content was 59.30-84.31%, ash content was 0.96-1.53%, dietary fibre content was 6.43-1.83% and oil content was 59.44-75.32%. The physicochemical properties of the oil samples ranged as follows: specific gravity 0.85-0.92; acid values 0.18-0.98 mg KOH/g; saponification value 123.42-187 mg KOH/g; iodine value 54-107g I_2/100g; and peroxide values 0.9-2.3 Meq O_2/kg. Altitude significantly influenced the proximate and physicochemical properties of avocado oil across different cultivars. Understanding these variations is crucial for optimizing avocado cultivation, processing, and utilization for nutritional and industrial applications.

Keywords Avocado, Cultivar, Altitude, Proximate composition, Physicochemical properties

1. Introduction

Since ancient times, plants have been an essential source of nourishment, providing vital nutrients that sustain human life while also serving as powerful tools for healthcare and disease treatment [1-12]. Beyond their role in food, plants contain a wealth of secondary metabolites-bioactive compounds that exhibit diverse pharmaceutical applications [13-21]. Many of these natural substances have paved the way for groundbreaking drug discoveries, offering potential leads for the development of new medications to combat infections and other ailments [22-26]. As science continues to explore the vast biochemical arsenal found in nature, plants remain invaluable in shaping both nutrition and modern medicine [27-38].

Persea americana Mill., commonly known as avocado, is a tropical evergreen fruit belonging to the Lauraceae family. It is classified as a climacteric fruit, meaning it continues to ripen after harvesting due to ethylene production. The fruit has generated great interest in recent years as a natural functional food due to its high nutritional value and health benefits. Avocado has a high nutritional value with a high content of unsaturated fatty acids, vitamins, phytochemicals, fiber, protein, and minerals such as magnesium and potassium [39,40]. Avocado fruits should be allowed to maintain sufficient maturity to be palatable upon ripening [41,42]. The question of when to start harvesting avocado fruit is of great commercial importance. It is difficult to determine the harvesting period especially for green-skinned varieties [43,44]. Maturity of avocado in several countries is determined by chemical analysis. Several researchers have studied different components of avocado pulp in order to determine their quality. Some of the components studied include maturity index, oil content, digestibility coefficient and amount of minerals in the pulp. These components have variations from season to season for each cultivar, geographical growing area, maturity, soil conditions and farm management practices.

Moisture or dry matter content determination has been the most common indicator used for avocado fruit maturity. However, other complementary indices can be considered, such as flesh softening and change of skin colour from green to black in some cultivars such as Hass. Dry matter is dependent on variety, region and season. This is a more accurate method of determining the maturity of avocados.

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24% and 30% dry matter are the minimum specification for fresh fruits maturity and for oil processing respectively in Kenya. Dry matter increases as the fruits mature [45-47].

Percentage oil content is another common indicator used for avocado fruit maturity [48]. Starting from 1925, a minimum standard of 8% oil content in the pulp of avocado fruits was used in the California avocado industry in the United States but since the eighties, they began using minimum oil content percentages for each cultivar: 10.0% for Fuerte and 11.2% for Hass [41,47,49]. The amount of oil depends on cultivar, maturity, geographical growing area, ripening stage and extraction method [50-52]. In a study of fatty content of three cultivars namely *Hass, Breda* and *Margarida*, there was significant difference in the percentage oil content between cultivars and different ripening stages [45]. In another study, fatty acid composition of oils from four avocado cultivars namely *Quintal, Fortuna, Margarida and Hass* similar observations were made [53]. Avocado harvested in early, mid and late season yielded significantly different percentage oil content [54].

Dietary fibre (DF) is found in wholegrain cereals, fruit and vegetables [55]. Fibre is made up of the indigestible parts or compounds of plants, which pass relatively unchanged through the stomach and intestines. Fibre is mainly a carbohydrate. The main role of fibre is to keep the digestive system healthy. Diets, deficient in DF, lead to a number of diseases such as constipation, hiatus hernia, appendicitis, diabetes, obesity, coronary heart diseases and gallstones [56]. Consumption of adequate amounts of DF reduces the risk of above-mentioned diseases [57]. Studies have shown that avocado oil has a high digestibility coefficient greater than 93.8%. Dietary fibre content varies with cultivar and the part of the fruit analyzed and geographical growing area [41,58].

Physicochemical properties of an oil are an important variable in considering its applications since physicochemical properties influence oil quality. Physicochemical properties like acid value, iodine value, saponification value, peroxide value provide information in determining the suitability of oil for consumption [59]. Further, when designing technological processes physicochemical properties of oil are important parameters to manufacturers [60].

Oils contain various triglycerides in different proportions. Specific gravity and viscosity of oil depend on the type of triglycerides present [53,61]. Specific gravity and viscosity decrease with increase in saturation and increases with unsaturation and polymerization [50]. 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. Refractive index is widely used in quality control to check for the purity of materials and to follow hydrogenation and isomerization of an oil [62].

Acid value of an oil indicates 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 [63]. 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 responsible for the stability [64]. 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 [65]. Additionally, oils with a noticeable acidity level are a sign that the plant may be toxic to cattle [66].

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 [66]. 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 [67,68]. The process of saponification involves treating a neutral fat with an alkali to break it down into glycerol and fatty acids (Figure 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 [69,70]. Peroxide value (PV) indicates the degree of lipid oxidation and hence its quality. It measures the peroxides formed during oxidation of a lipid. High PV values indicate high levels of oxidative rancidity in the oils as well as the lack or insufficiency of antioxidants such as propyl gallate and butyl hydroxyl anisole which are used to reduce rancidity [68,71]. A maximum allowed peroxide level of 10 mg equivalent of oxygen/kg of oils was established by the WHO/FAO [72].

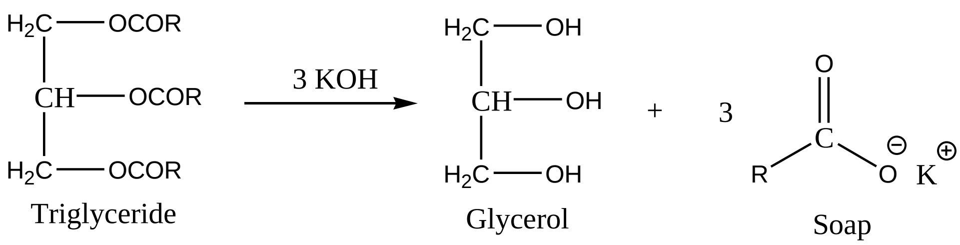

Figure 1. Saponification of natural fat

2. Materials and Methods

Sampling and sample preparation

Mature avocado fruits were picked from different farms selected randomly and purposively from the three different climatic zones In Murang'a County. The farms from the upper zone were distributed above 2200m above sea level (asl) in Kanderendu area, Kangari Ward in Kigumo constituency on coordinates (0°47'S, 36°49'E). The farms from the middle zone were distributed between 1700-2200m asl in Gatanga and Kihumbu-ini wards in Gatanga constituency on coordinates (0°55'S, 36°57'E). The farms from the lower zone were distributed between 1300-1550m asl in Ichagaki and Kamahuha areas, Maragua constituency on coordinates (0° 47'S, 36º49'E). The samples were identified and labelled accordingly. The samples were sorted and kept for 4-7 days to ripen. The ripe fruits were thoroughly washed with 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 removed and the pulp cut into small pieces and crushed in a mortar.

Lipids Extraction

The extraction of oil was done in a Soxhlet extractor using petroleum ether using the method described by Manaf et al [73]. A homogenous sample was dried in an oven for 24 hours at 70℃ then ground into fine powder. 10g of the dried sample was mixed with petroleum ether. The mixture was transferred into a Soxhlet extractor for 6-8 hours at 40-60℃. The extract was then evaporated in a rotary evaporator at 95℃ to evaporate the solvent. The lipid was weighed and expressed in % w/w on the dried fruit weight basis.

Moisture Content

Moisture content was determined by the method described by Carvalho et al [45] as follows; Two grams of the sample was accurately weighed in clean dry crucible (W_1). The crucible was oven dried at 100 -105℃ until a constant weight was obtained. The crucible was then placed in the desiccator for 30 min to cool. After cooling, it was weighed again (W_2) .The percent moisture was calculated using the formula below:

$$\% \text{ Moisture} = (W_1\text{-}W_2)/W_0 \times 100$$

Where W_0 is Weight of sample (g), W_1 is Initial weight of crucible + Sample (g), W_2 is Final weight of crucible + Sample (g).

Ash Content

An empty crucible was weighed and the weight recorded as (W_0). Two grams of the sample was placed in the crucible and the weight recorded as (W_1). The crucible was placed in a furnace at 550℃ for 3 hours. The crucible was allowed to cool and weight recorded as (W_2). The percentage of the ash content was calculated using the formula below;

$$\% \text{ Ash content} = (W_2\text{-}W_0)/(W_1\text{-}W_0)\times 100$$

Where W_0 is weight of empty crucible (g), W_1 is weight of crucible + powdered sample (g), W_2 is weight of crucible + ash sample (g).

Dietary Fiber

Crude fibre was determined by the method described by Maitera et al 2014 as follows: 5g sample was digested into trichloroacetic acid for 40 min. The residue was filtered and washed with boiling water and acetone. The residue was heated at 105℃ in an oven. The residue was scrapped and weighed (W_1), then ashed in a furnace at 550℃ for 2 hours. The sample was then cooled and weighed (W_2). The fibre content was calculated using the formula below.

$$\%\text{Fibre} = (W_1\text{-}W_2)/W_0{*}100$$

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

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 [74].

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 25℃ 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 [74].

Specific gravity= z-x/ y-x

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

Acid Value

Acid value was determined by the method described by Nasri *et al*. [52] 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

$$\text{Acid value} = 56.1V{*}N / W \text{ (mg of KOH/g of oil)}$$

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

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 1000ml 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 [74].

$$\text{Saponification value} = (V_B\text{-}V_W) *56.1N/W \quad (\text{mg KOH/g of oil})$$

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

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 1000ml in a volumetric flask. The solution was then standardized using 0.01N potassium dichromate solution [74]. The normality of the sodium thiosulphate was then determined by the formula;

$$F = 20 \times F'/Vs\text{-}Vb$$

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 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*. [52] as follows: 0.4g of the oil sample was accurately weighed into a conical flask. 20ml 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 20ml of 10% KI with 125ml of water was added. The solution was then titrated with 0.01N $Na_2S_2O_3$ using starch indicator until the blue black coloration changed to colourless. Blank determination was done without oil.

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

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.

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 10ml of a solvent made by mixing glacial acetic acid and chloroform in the ratio 3:2. 1ml of saturated KI (prepared by dissolving 100 g of KI in 70ml of distilled water) was added to the sample and the mixture was immediately stoppered and store in the dark for 5 minutes. 20ml 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 [74].

$$PV \text{ (meq of } O_2\text{/kg of oil)} = (Vs-Vb) \times F \times N \times 1000 /W$$

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. Results and Discussion

Proximate Composition

Fuerte grown in the zone 3 had the lowest moisture content of 59.30% while Pinkerton grown in zone 1 had the highest moisture content of 84.32%. The moisture content was significantly different between cultivar and geographical growing environment (Figure 2). 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 [45]. 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 this criteria except Pinkerton grown in zone 1 with a dry matter content of about 16%. From these results, avocado fruits contain high moisture content and therefore they have shorter shelf-life. The moisture content values are similar to those reported from other studies [49].

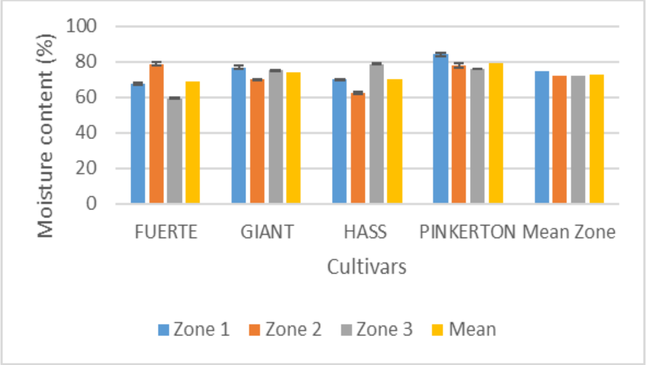

Figure 2. Variation of moisture content with cultivar and zone

The oil content of avocado grown in Murang'a County ranged between 59.44-76.06% on dry weight basis (Figure 3). Fuerte had the highest oil content in all the zones followed by Hass, Giant and Pinkerton at 74.63, 70.46, 68.62 and 63.67% respectively. Zone 2 yielded the highest amount of oil for Fuerte (76.06%), Hass (74.82%) and Giant (71.53%). For Pinkerton the highest yield was in zone 1 (67.38%). The amount of oil was lowest in all the cultivars studied in zone 3. The percentage oil content was significantly different between cultivar and geographical growing environment (zone).

These values obtained in this study were higher than those reported earlier in Indonesia, Nigeria and Colombia [45,58,73]. The percentage of oil content varied with cultivar and geographical growing area. There was great correlation between moisture content and percentage oil content allowing the later to be used as an indicator for avocado maturity [41].

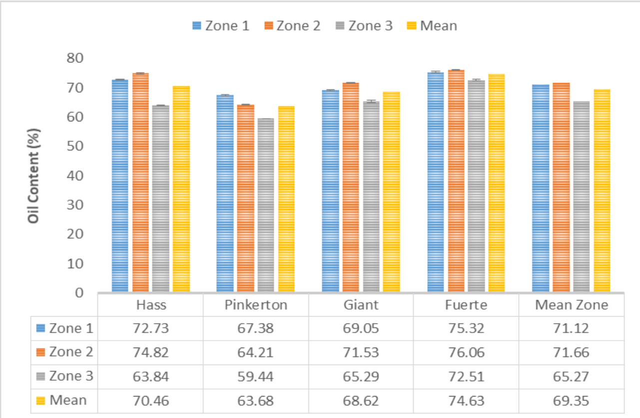

Figure 3. Effect cultivar and zone on oil content of avocado pulp

The ash content ranged from 0.63% to 1.60% (Figure 4). The lowest ash content was reported in Hass grown in zone 2 (0.63%) while the highest was reported in Giant cultivar grown in zone 3 (1.60%). The Giant cultivar had the highest ash content in all the zones while Hass had the lowest. The ash content increased linearly in Pinkerton and Giant from zone 1 to zone 3, whereas in Hass and Fuerte it was highest in zone 1, decreased in zone 2 and then increased in zone 3. All the cultivars had the highest ash content in zone 3. Giant cultivar registered the highest ash content followed by Pinkerton, Fuerte and Hass. These results were similar to those previously reported by some researchers [41,76] but lower than those obtained by from other studies [58]. Ash content is related to the level of minerals in the avocado samples [78].

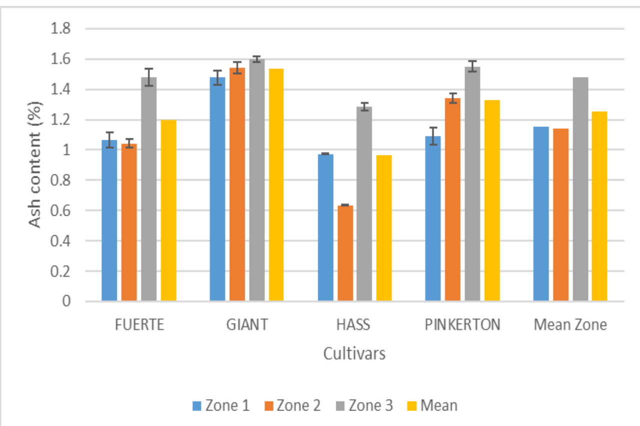

Figure 4. Effect cultivar and zone on ash content of avocado pulp

Dietary fibre content in the samples ranged from 1.83 to 6.43% (Figure 5). Pinkerton cultivar had the highest dietary fibre content in all zones followed by Giant, Hass and Fuerte in that order except in zone 3 where Fuerte had higher dietary fibre content than Hass. The dietary fibre content was highest in zone 1 and lowest in zone 3. The dietary fibre was significantly different between cultivar and geographical growing environment (zone). These results are similar to those obtained by other researchers [41].

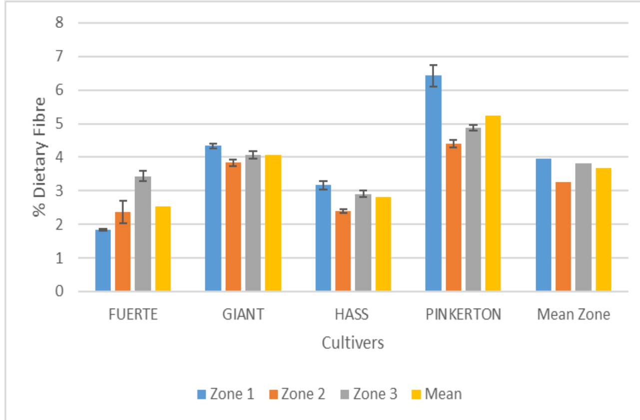

Figure 5. Variation of dietary fibre content of avocado pulp with cultivar and zone

Physicochemical Properties of Oils from Avocado Cultivars

Specific gravity ranged from 0.91 to 0.85 (Figure 6). 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. The results are compared to those obtained in avocado samples by other [4,58,79].

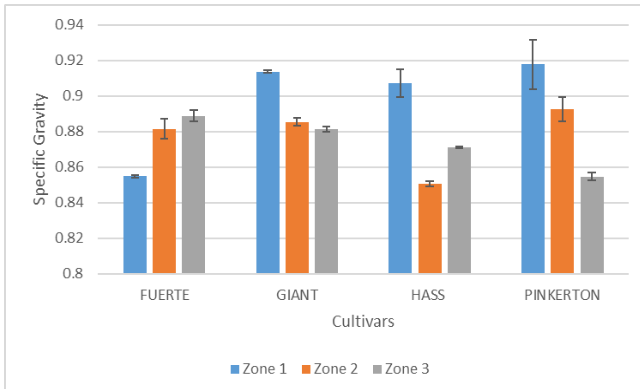

Figure 6. Variation of specific gravity of avocado oil with cultivar and zone

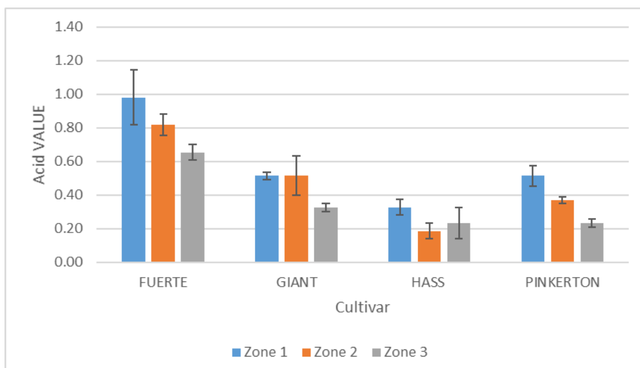

Figure 7. Variation of acid value of avocado oil with cultivar and zone

The acid values of the avocado oil samples range from 0.18mg of KOH/g of oil to 0.98mg of KOH/g of oil (Figure 7).

Fuerte cultivar had the highest acid value in all the zones while Hass has the lowest. The 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 mg of KOH/g of oil for the pulp oil of the Fortuna, Collinson, and Barker cultivars, respectively [41]. The acid values were lower than 0.186 obtained in Nigeria [58].

The iodine value of the oils ranged from 54.3g/100g of oil to 107.3g/100g of oil (Figure 8). These values were within the range reported in other parts of world [50,58,61]. The iodine values of avocado collected from zone 1 were 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 the highest iodine value compared to the other cultivars in this study. High iodine value indicates that Hass avocado oil contains the highest composition of unsaturated fatty acid [50]. There was significant difference in the iodine values observed between the cultivars and the zones.

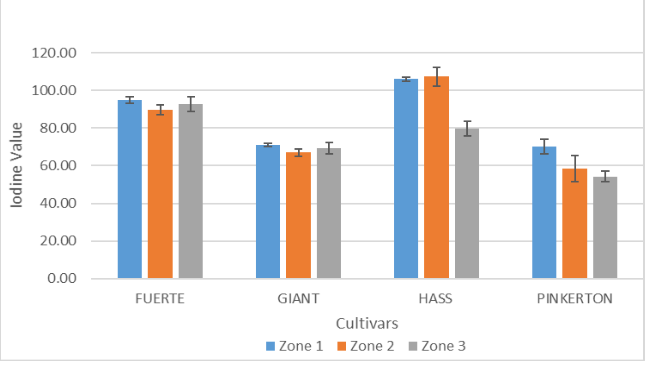

Figure 8. Variation of iodine value of avocado oil with cultivar and zone

The saponification value of the samples ranged from 123.42 mg of KOH/g of oil to 187 mg of KOH/g of oil (Figure 9). On average, avocado fruits collected from zone 1 had a higher saponification value (151.00) while those from zone 2 had the lowest saponification value (144.92). There were significant differences among the cultivars and zones.

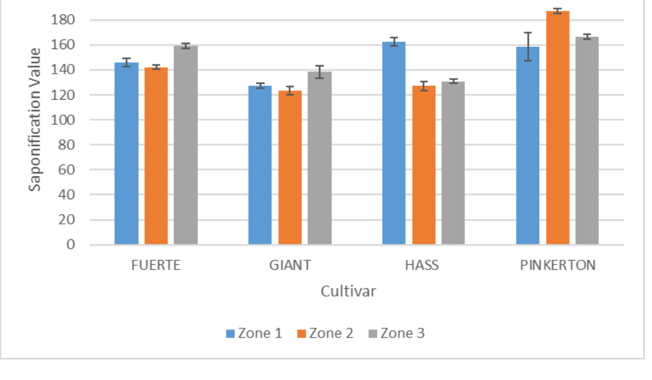

Figure 9. Variation of saponification value of avocado oil with cultivar and zone

The peroxide values ranged from 0.9meq O_2/kg fat to 2.3meq O_2/kg fat (Figure 10). Fuerte avocado oil had relatively higher peroxide value compared to the other cultivars, indicating the oil is unstable against oxidation. Pinkerton had the lowest peroxide values indicating the oil is stable against oxidation. These values are similar to those reported in other studies (2.05-2.6) [41]. The values reported varied with geographical growing environment, cultivar and soil composition.

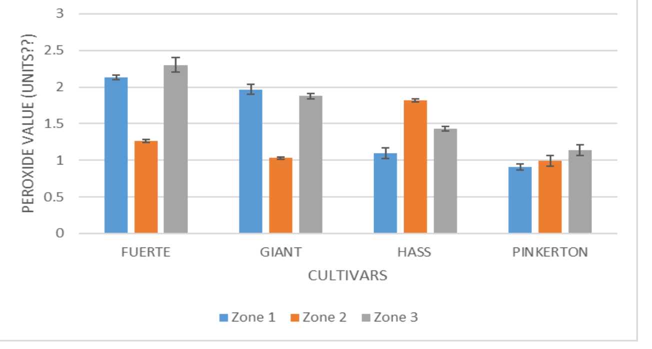

Figure 10. Peroxide value of oils from different cultivars and zones

4. Conclusions

This study aimed to investigate the influence of the cultivar and altitude on the proximate properties including the moisture, ash, oil and dietary fibre contents of avocado cultivars grown in Murang'a County. The physicochemical properties of oil from the avocado samples including specific gravity, acid value, saponification value, iodine value and peroxide values were also investigated. This study highlights the influence of altitude on the proximate composition of avocado pulp and the physicochemical properties of avocado oil across different cultivars. The findings suggest that altitude plays a significant role in determining nutrient content, oil yield, and quality parameters. Understanding these variations is crucial for optimizing avocado cultivation, processing, and utilization for nutritional and industrial applications. Further research could explore the mechanisms behind these differences, helping to enhance avocado-derived products for both local and global markets.

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