NUTRIENT COMPOSITION OF AVOCADOS GROWN IN HAWAI`I AND CAMEROON

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Abstract

Avocados (*Persea americana*) are a source of monounsaturated fatty acids, dietary fiber, and beneficial phytochemicals. Despite the health benefits of avocados, there is limited nutritional research on Hawaii and Cameroon-grown avocados. The health benefits of the fruit are primarily based on research involving the Hass cultivar. In Hawaii and Cameroon, many other avocado cultivars besides Hass are consumed. The objective of this research is to illustrate the nutrient content variability among the common varieties of Hawaii and Cameroon avocados. In doing so, the data will help consumers make informative decisions when purchasing the fruit. The data will also help decrease the deficiency of nutritional information which may restrict the growth of the local avocado industry in Hawaii and Cameroon. If consumers know the nutrient profile of avocados, they might be more apt to buy locally grown fruits rather than imported fruits.

The nutritional quantities of six Hawaii-grown cultivars and four Cameroon-grown cultivars were analyzed. The Hawaii cultivars were Linda, Beshore, Serpa, Nishikawa, Ohata and Murashige. The Cameroon cultivars were Peteson, Pollock, Fuer Florida and Booth VIII. Fatty acids (FA) were extracted via Soxhlet, then hydrolyzed and quantified via LCMS. Total protein content was determined via Kjeldahl digestion, mineral profile via ICP, and carotenoid content via HPLC. Nutritional profiles varied among the cultivars. The genetic background appeared to have a stronger influence than the environmental and growing factors as cultivars form the same farms differed from each other.

This data will provide information on the most suitable cultivars in a nutritive and commercial perspective. Furthermore, the public in Hawaii and Cameroon will benefit from the nutritional information on avocados grown in their local region. This will also help improve understanding the health benefits of the fruit which are related to their chemical composition.

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Chapter 1

Literature Review: Avocados

1.1 Avocado *Persea americana*

Avocados are a fruit in the Lauraceae family. Specifically, the fruit is a single seeded berry with the scientific name *Persea americana*. Avocados have a circular appearance, either being round, oval or tear-shaped. The latter appearance results in avocados being called “pears”, resembling the fruit pear (genus *Pyrusl*). The shape of an avocado is cultivar specific. The length of an avocado fruit ranges from 7 – 33cm with a width of 7 – 15cm (Bill *et al*., 2014). The average weight of an avocado ranges from 140 – 1000g (dos Santo *et al*., 2014; USDA, 2016). The variability in the size and shape can be seen among the many different cultivars. For example, the common Hass cultivar has an average weight range of 140 – 400g with an average length and width of 7cm and 6cm. The Ohata variety weights between 800 – 1000g with an average length and width of 17cm and 11cm (Love, 2009).

Figure 2. Images of Hawaii grown cultivars. From left to right: Hass and Ohata. (Ken Love, www.hawaiifruit.net).

Avocados can be described by their three distinct portions or layers. The outer layer is known as the skin or peel and resembles a shell, a leathery coat protecting the flesh and seed. The color of the skin ranges from dark green to light green to purple and black. The texture of the peel can be smooth or bumpy with varying degrees of thickness. Both the skin color and texture varies among the cultivars with no apparent trend to predict the outcome. The inner flesh of the fruit is called the mesocarp. This portion is the most edible part of the fruit. The color of the mesocarp can be a mix of yellows and greens.

The seed is found towards the middle of the fruit and tends to be circular, conforming to the general shape of the whole fruit. Different cultivars have different seed length, but generally the seed is 5 – 7cm long (Bill *et al.*, 2014). On average, the seed and peel comprise 25 – 33% of the total weight of the whole fruit (Dreher and Davenport, 2013; USDA, 2016).

Avocados are prized for their unique flavor. Although the peel and seed have nutrients, these are generally not eaten. The mesocarp tends to be agreeable to consumers due to the rich creamy taste. The butter-like texture is due to the oil in the meat of the fruit. Some cultivars have been described as having a nutty taste (McDonald, 2010). Research done in Hawaii revealed the local public, both cooking professionals (i.e. chefs) and non-professionals, prefer the mesocarp to be thicker and smoother rather than watery and fibrous (Barber *et al*., 2008). The research also found consumer preference towards the cultivars with a higher edible flesh-to-seed ratio. Of the Hawaii grown commercial avocados, the Sharwill cultivar had the smallest seed (McDonald, 2010).

The oil in avocados is largely found in the mesocarp of the fruit. Located in this portion of the fruit are parenchyma cells, idioblast cells and a network of vascular strands (Mostert *et al*., 2007). The large parenchyma cells have numerous droplets of lipid substances (e.g. triacylglycerol) in them. The idioblast cells on the other hand, usually have single droplets of oil in their oil sacs. Parenchyma cellular oil and idioblast cellular oil differ in fatty acid composition (Mostert *et al*., 2007). The maturity of the fruit is closely associated with the oil content in the fruit. The amount of oil is used as an indicator of when to harvest.

Figure 2. Images of Hawaii grown and Cameroon grown avocado cultivars. From left to right: Nishikawa, Peterson, Pollock, and Peterson cultivars. (Ken Love, www.hawaiifruit.net).

1.2 History of *Persea americana*

Avocado is the English name of the fruit and tree. This name is adapted from the Spanish name aguacate or ahuacate. The Spanish derived their name from the Aztec name for the same fruit, ahuacatl (Bost, 2013). The first emergence of the avocado tree and fruit dates back nearly 10,000 years ago to 8000 – 7000 BC. Fossils of seeds were found in ancient human settlements in what is known today as Puebla, Mexico (Chen *et al*., 2009). Historians believe the domestication of avocados, maize, and beans helped transition the early settlers from a nomadic hunter-gather lifestyle to a domestic agricultural-based life. The avocado was so impactful in the lives of ancient Mayans that the season of the fruit was represented in the 14th month of their civil calendar. The names of the months in the Mayan calendar were based on notable seasonal and agricultural events (Galindo-Tovar *et al*., 2007). Inscriptions of avocado trees were also found in tombs dating back to 650 AD. It was believed the dead returned to the earth in the form of plants to help ensure the survival of their loved ones (Galindo-Tovar *et al*., 2007).

The first written description of the avocado was in 1519 by the Spanish explorer, Martín Fernández de Enciso, who likened the fruit's appearance to an orange, and its taste to butter (Galindo-Tovar *et al*., 2007). He noted the flesh was yellow in color and had a very pleasing and good flavor. Towards the end of the 16th century, scientist Francisco Hernandez introduced the fruit to King Felipe II of Spain and the Indies. The king likened the tree to an oak tree with black fruits, like figs, hanging from its branches. The king was also told the avocado tree was abundant and could be found almost everywhere in the new world (Galindo-Tovar *et al*., 2007). The Spanish explorers enjoyed the avocado and brought it with them as they navigated the world. By 1750 Indonesia had the avocado tree, then in 1809 Brazil was introduced to it and in 1890 the Philippines started growing avocados (Bost, 2013).

The early settlers as well as the Spanish explorers, valued avocados for their nutritional value and their folk remedies. The fruit was used to help with skin abrasions and for hair treatments, particularly growth (Galindo-Tovar *et al*., 2007). The fruit was made into a paste and applied to hair to help prevent damage and splitting. In Mexican folk medicine, the avocado leaves helped alleviate coughs. Other New World medicinal uses of the fruit include dysentery and diarrhea treatment. Mayans believed the seed would alleviate dry inflamed skin, and would rub it on affected areas. They also

believed eating the fruit would promote healthy blood (Galindo-Tovar *et al*., 2007). Given the health benefits of avocados, many of these ancient uses are supported by modern research. The seed of the avocado does have oil content with biochemical properties supportive of tissue remodeling and wound healing (Mokady and Neeman, 1990).

Research has pinpointed the domestication of the avocado to three distinct geographic locations which has resulted in three distinct horticultural races (Bill *et al*., 2014; Chen *et al*., 2009). The three avocado ecotypes are Mexican, Guatemalan and West Indies. The West Indian race is a tropical lowland avocado, while the Mexican and Guatemalan races are tropical highland, with the difference being the Mexican is cool subtropical while the Guatemalan is warm subtropical (Bost *et al*., 2013). Chen *et al*. (2009) point out the names are misleading as the West Indies avocado does not occur in the West Indies. Since its domestication, the avocado has been hybridized resulting in the many contemporary cultivars seen today. The cross breeding is both spontaneous and purposeful, with the latter being done to produce high quality marketable fruit. The California grown avocados are hybrids of Guatemalan and Mexican horticultural races whereas the Florida grown avocados are hybrids of Guatemalan and West Indies races (Chen *et al*., 2009). The spread of the avocado agricultural industry world-wide exasperates the degree of emerging avocado cultivars. This is nowhere more evident than in the unique cultivars grown in Hawaii over the past 200 years (Love, 2009).

1.3 Cultivation and Harvest

Avocados are considered by many horticulturalists to be a young crop, having only been an orchard crop since the early twentieth century (Wolstenholme, 2013). However, in a relatively short amount of time avocados have earned the distinction of being the fourth most important tropical fruit in the world (Galindo-Tovar *et al*., 2007). Currently the largest producers of the fruit are Mexico, Colombia and Peru (FAOSTAT, 2017). In 2014, Mexico produced nearly a fourth of the world's avocados at 1.5 metric tons. As natives of Central America, the avocado thrives in warm, humid tropical to subtropical regions. A desirable attribute of avocados is their adaptability. They grow in elevations from sea level to over 2500m. Avocados have been found in the Mexican highlands at 1400 – 2700m; in Guatemala they grow between 1500 – 2350m and in Colombia at 100 – 450m (Wolstenholme, 2013). In Hawaii, they

thrive at elevations from sea level to about 600m on coffee plantations (McDonald, 2010). The greatest influence on both trees and fruits are temperature and water. The ideal temperature range for most avocados is 16 – 30°C. This however varies among the cultivars, with some varieties able to grow at -7°C with minimal damage (Schaffer, 2013). Avocados do not tolerate freezing conditions, at such cold temperatures it's likely the tree will not grow and produce both flowers and fruits.

Avocado trees grow in areas with as little as 18cm of rainfall (i.e. semi-desert, irrigated area) to areas with over 203cm of rain (Wolstenholme, 2013). Despite their versatility, the plant thrives with little maintenance in humid to semi-humid environments. In the Mexican highlands (semi-tropical, semi-humid) where avocados originate from, the average annual rainfall is 91cm with temperatures around 16°C. In the Guatemalan tropical highlands, avocados grow with an average rainfall of 140cm with temperatures in the 20s°C. In the semi-humid tropical lowlands of Colombia, annual rainfall ranged from 100 – 152cm with temperatures in the high 20s°C (Wolstenholme, 2013). According to Chia *et al*. (1987), 102 – 127cm of rain per year is a sufficient quantity of water for the tree in temperatures ranging in the high 20s°C. Abundant rain during the flowering phase which usually takes place in the dry seasons will prolong the flowering period and delay fruit production.

Areas of drought and general high level stresses (i.e. cold/heat stress, salinity stress, wind stress) typical in cool semi-arid areas will result in decreased yield. In higher temperatures, increased water is needed due to evaporation and over-heating. However too much water is harmful to the tree as avocados need well aerated soil. The tree is susceptible to root rot disease (*Phytopthera*) which occurs with high rainfall and poorly drained soil (McDonald, 2010). Since the pathogen did not naturally occur in the native habitats of avocados, the plant did not evolve a resistance to this damaging disease (Schaffer *et al*, 2013). A vegetative strategy of the avocado plant is its shallow inefficient fibrous feeder roots that require a large amount of oxygen, hence the need for porous soil. Avocado trees can somewhat compensate their fruit quality in stressful environments by efficiently intercepting, hoarding and recycling limited nutrient resources (Schaffer, 2013).

Avocado trees are highly adaptable to their surroundings, evolving into different species (Galindo-Tovar *et al*., 2007; McDonald, 2010). Physically, the trees are tall with many far-reaching limbs. The

estimated average height of an avocado tree is between 12 – 24m. Regardless of the variety, the tree needs to grow in wind protected locations as the wood of the truck and branches is brittle and prone to breaking. This is a common trait among all cultivars. High winds are also damaging to both flower and fruit. This particularity makes a region like South Kona on the Island of Hawaii an ideal growing location. There are two large active volcanos, Mauna Loa and Hualalai that provide natural protection from the trade winds. Avocado trees will thrive with good secure windbreakers (McDonald, 2010).

Avocados are a self-pollinating tree, albeit only partially. In commercial production, the tree is propagated by grafting, in order to have a predictable quality and quantity of fruit yield. The reproductive biology of avocados is fundamental to increasing flowering, fruit set and yield while reducing the cost of growing inputs (e.g. water, fertilizer, land) (Salazar-Garcia *et al*., 2013). Avocado trees have an abundance (100,000+) of small complete and perfect flowers, however many of them do not set fruits (Salazar-Garcia *et al*., 2013). The abundance of flowers increases the likelihood of pollination and the flower size makes it accessible to small insects. The open-pollination and interracial hybridization have given rise to the common cultivars seen today. For example, the Hass and Bacon cultivars are hybrids of the Guatemalan and Mexican horticultural races (Bill *et al*., 2014). The avocado has a unique flowering mechanism known as synchronous protogynous dichogamy, which describes the male and female organs being separated temporally (Salazar-Garcia *et al.,* 2013). The time of bloom of male and female flowers is not uniform among the many cultivars; such a difference within a fruit species is unusual.

There are two types of avocados (i.e. A and B) based on their flowering phases. Avocado type A cultivars have flowers that open functionally as females first (i.e. day one of anthesis) in the morning and close in the early afternoon on the same day. The next day (i.e. day two), the flowers will open as male flowers in the afternoon. Cultivars that are A include Hass, Gwen, and Reed (Salazar-Garcia *et al.,* 2013). Avocado type B cultivars have flowers that open functionally females as in the afternoon on the first day of the flowering period and close in the late afternoon on the same day. The flower then re-open the next morning (i.e. day two) as functional males. Cultivars that are B include Sharwil, Fuerte, and Bacon (Salazar-Garcia *et al.,* 2013). The time of flowering is determined by genetics and climate conditions and is crucial to a profitable crop. Blooms that open in the late winter/early spring are at risk

for low pollen development and/or fruit set from the low temperatures. Blooms that open in the late spring/early summer may be adversely affected by the high temperatures. Rain also affects reproduction and fruit development by hindering insect activity and flower phenology.

Avocados are unique among the fruit tree crops in the time it takes for fruit development, which is significantly longer. Generally, fruits may be harvested 8 – 22 months after peak bloom, depending on the growing conditions and environmental stresses (Wolstenholme, 2013). The popular Hass cultivar was observed to reach maturity 14 – 18 months after the first bloom in cool regions. The growing of avocado fruits is further complicated by the low fruit set. Even though there is an abscission of flowers and immature fruits, only a very small amount will persist to harvest. Fruit set was found to be less than 0.1% in healthy, well-managed orchards. The large loss of fruits is a self-regulatory mechanism typical of most forest species and fruit tree crops. This natural thinning process however limits the avocado industry and underlines the need to determine the tree's energy requirements for a larger crop. The trees are physiologically capable of yielding more fruit and with the agricultural technologies applied to fruits like the apple and orange, it's feasible. Industry expects project an avocado orchard may produce an average of 32.5 tonnes per hectare of fruits with 17% oil content (Wolstenholme, 2013).

One observation of the avocado fruit crop is the irregularity of fruit production. The Hass cultivar is known to have alternating low yield seasons. Crop load is correlated to external factors such cold temperatures and availability of resources. Although biennial bearing is not guaranteed, it is common and causes concern in the agricultural industry (Schaffer, 2013). To compensate for potentially low yield seasons, farmers devote large acreages to avocado trees. According to the Food and Agriculture Organization of the United Nations (2017), Mexico has over 150,000ha of commercially producing trees. Orchard densities range from 200 – 2000 trees per hectare (Schaffer, 2013). The average spatial arrangement in an avocado orchard is 20x20ft. The range in spacing is reflective of the robustness of the plants due to environmental conditions, growing techniques, and avocado variety. In warm, humid subtropical climates such as Florida, parts of Mexico and Brazil the trees grow spread out, needing larger spatial distance. In semi-arid and arid climates, such as California, trees are less vigorous and can be placed closer together (Schaffer, 2013).

The avocado fruit is considered a climacteric fruit. This means the fruit ripens with increased ethylene production and bursts of cellular respiration. Other climacteric fruits include bananas, apples and tomatoes. Although the fruit matures on the tree, it ripens after being harvested. During the ripening stage, the walls of the parenchyma cells degrade due to cellular enzymes (i.e. cellulose and polygalacturonase). It is from this enzymatic activity that the oil is released from its cellular storage. Research has identified parenchyma cells to be susceptible to cellular enzymes while idioblast cells are immune and remain intact throughout the ripening stage (Mostert *et al*., 2007). Oil content does not increase after harvest. This underlies the importance of harvest; knowing when to pick an avocado is crucial in providing the consumer with the best quality fruit. There is a trade-off between picking too late with high oil content and too early with a fruit that is easily transported. Mature, ripe avocados tend to be soft and susceptible to damage, while immature unripe avocados are firm and can withstand rough handling but may have lower oil content. Low oil content may result in poor sales and revenue loss. Furthermore, the ratio of saturated fat to unsaturated fat decreases as avocados ripen; the change in the fatty acid composition is postulated to influence the degree to which an avocado benefits the consumer's health (Dreher and Davenport, 2013).

1.4 Nutrient Composition

Avocados are an energy-rich food eaten in its fresh state. The nutrient content of avocados varies according to the cultivar, season, growing conditions and time of harvest (Lu *et al*., 2009; Naveh *et al*., 2001). The time when an avocado is picked (i.e. the time of harvest) is crucial in the development of the fruit and therefore the nutrients present. Avocados mature on the tree and if harvested too early in its maturation stage the nutrient composition will be less than optimal. This is particularly the case for lipid content as the oil increases during maturation. (Lu *et al*., 2009). From a botany perspective, oil-storing is an energy-intensive process for the plant and a reason for the variability among the many cultivars of avocados (Schaffer, 2013). Avocados are further distinguished from other fruit tree crops, in that this fruit accumulates high concentrations of protein in the mesocarp (Salazar-Garcia *et al.,* 2013).

The USDA database is considered the complete reference for nutritional information on food

items in the American marketplace. A search for raw avocados in the database returned with three entries: California avocados, Florida avocados, and all commercial avocados. Since majority of avocados are imported from around the world, the "all commercial varieties" nutrient report seemed most relevant to the variety of avocado normally purchased from a store. According to USDA (2016), there is 160kcal in 100g of raw avocado, however no data points were reported. Protein was found to be 2g per 100g raw wt. and total lipid was 14.66g/100g fresh wt. The Recommended Dietary Allowances (RDAs) are the average daily amounts of nutrient intakes adequate to fulfill the needs for most (i.e. 98%) of the healthy population. These dietary reference intakes are referred to by many health professionals when advising a nutritious diet. Healthy adult males and females ages 19yrs and older are recommended to consume 0.80g of protein per kg of body weight every day (Gropper and Smith, 2013). For example, a 180lb (82kg) middle aged man needs 66g of protein each day. There is roughly 9g of protein in a pound of edible avocado. Avocados can be a healthy way to provide the body with additional protein and complement complete protein sources such as fish, eggs and poultry. Protein comprises an estimated 20% of the human body, highlighting the importance of this nutrient in creating and maintaining a healthy physical and mental status. Understanding this is crucial when designing meals with the highest bioavailable amino acids.

The carbohydrate content was determined by the difference in the weights of the total fruit and individual weights of crude protein, fat, dietary fiber and mineral content. The USDA nutrient database (2016) reports the carbohydrate content in avocados is 8.53g/100g fresh wt. Total dietary fiber, which includes both soluble and insoluble, is 6.7g/100g fresh wt., and total sugar is 0.66g/100g fresh wt. (USDA, 2016). These nutrient content values in the USDA database were generally seen in the reported literature. Data from Naveh *et al*. (2001) found dietary fiber content in fresh avocados to be 5.2g/100g fresh wt. Dreher and Davenport (2013) reported the fruit's carbohydrates were 80% dietary fiber and 20% sugars. The dietary fiber was 70% insoluble and 30% soluble. Based on these findings, eating a half of an avocado provides 4.2g of dietary fiber and 300kcals. The adequate intake for dietary fiber is 14g per 1000kcal (Gropper and Smith, 2013). A major sugar in raw avocados is D-mannoheptulose, a seven-carbon sugar that does not behave nutritionally like a conventional sugar and is believed to be a phytochemical unique to avocados (Dreher and Davenport, 2013). The overall sugar content is low

(0.66g/100g fresh wt.) and supports the avocado having a glycemic index and load around zero.

The RDA for carbohydrates for adult males and females 19yrs and older is 130g per day. Dietary fiber has an Adequate Intake (AI) reference value instead of an RDA. This is because of the insufficient clinical evidence to establish an amount of daily dietary fiber that will confer health benefits for majority of the healthy population. Adult males are advised to consume 38g a day of dietary fiber and adult females are advised to consume 25g a day. One pound of avocado mesocarp has about 38g of carbohydrates and 30g of dietary fiber. These values infer avocados are a healthy way to meet the daily references for both carbohydrates and dietary fiber. When examined with its protein and lipid content, the avocado is a nutrient dense food with potential health benefits.

1.5 Lipid Composition

Avocados are valued for their fatty acid content, deeming the fruit a high-energy food source. Many health benefits of the fruit stem from the unsaturated fatty acids present; these specific fats comprise roughly 84% of the total fat in a ripe avocado (Dreher and Davenport, 2013). There are two essential fatty acids required in the diet of humans; they are omega-6 fatty acid linoleic acid and omega-3 fatty acid α -linolenic acid, both of which are found in avocados. According to the USDA database (2016), 100g of raw avocado has 1.674g of linoleic acid and 0.111g of α -linolenic acid. Avocados grown in Japan were found to be 18 – 22% oil (Takenaga *et al*., 2008). Roughly 11% of the total lipid was linoleic acid and 0.8% was α -linolenic acid (Takenaga *et al*., 2008). Ripe Hass avocados were found to have 15.42% oil content with 15.41% being linoleic acid and 0.25% being α -linolenic acid (Ozdemir and Topuz, 2004). Ripe Fuerte avocados had 15.43% oil content with 12.13% being linoleic acid and 0.16% being α -linolenic acid (Ozdemir and Topuz, 2004). Oleic acid is a monounsaturated fatty acid predominate in avocados, being consistently reported in literature for the fruit (Landahl *et al*., 2009; Meyer *et al*., 2008; Moreno *et al*., 2003). USDA (2016) reports 9g of oleic acid in 100g of raw avocado. Palmitoleic acid is another monounsaturated fatty acid found in avocados, albeit very small at 0.698g per 100g fresh wt. (USDA, 2016). Ripe Hass avocados were found to have 15.42% oil content with 53% being oleic acid (Ozdemir and Topuz, 2004). Ripe Fuerte avocados had 15.43% oil content with 65% being oleic acid (Ozdemir and Topuz, 2004).

Total lipid content changes within a growing season, particularly as the fruit matures. Lu *et al*. (2009), correlated increased dry mater and oil content in avocados harvested late in their season. Overall though, fatty acid composition is significantly influenced by the variety, season, and growing conditions (e.g. climate and soil). The National Institutes of Health (2016), recommend 17g of linoleic acid a day for adult males and 12g a day for adult females. For α -linolenic acid, adult males are recommended to have 1.6d/day and adult females 1.1g/day (Gropper and Smith, 2013). The presence of both essential fatty acids along with other unsaturated fatty acids in avocados makes this fruit's consumption appropriate for human health, and may reduce the risk of diet related disease such as obesity, diabetes and heart disease.

1.6 Antioxidant and Carotenoid Composition

Avocados have bioactive molecules with protective properties against the harmful effects of free radicals (Jacobo-Velazquez and Hernandez-Brenes, 2012). These molecules are known as antioxidants and include carotenoids and vitamins C and E. Avocados also contain persenone A and B which are protective against inflammation and carcinogenesis (Jacobo-Velazquez and Hernandez-Brenes, 2012). Common among many plants are phytosterols which have a similar chemical structure to cholesterol present in the human body. Phytosterols resemble cholesterol just enough to reduce intestinal absorption of cholesterol via competitive binding and thereby decreasing the amount present in the blood stream (Lin *et al*.,2009; Racette *et al*., 2010). Avocados were found to have phytosterols in their mesocarp with the most abundant compound being beta-sitosterol, comprising 89% of the total phytosterol content (Plaza *et al*., 2009). Beta-sitosterol levels were found to be 89.18g in 100g of avocado pulp oil (dos Santos *et al*., 2014). An advantageous trait of avocados is the antioxidant content helps to minimize the loss of phytosterols during packaging and storing (Plaza *et al*., 2009). The skin and seed portions of the fruit, deemed inedible, were found to have higher antioxidant levels than the mesocarp which supports their role as preserving the beneficial phytochemicals in the edible portion (Rodrigez-Carpena *et al*., 2011).

Based on the pigmentation of avocado tissue and oil, there are carotenoids, flavonoids, and sterols among other bioactive compounds. The most common carotenoid in avocados is lutein. Lutein is a xanthophyll made only by plants and its color ranges from yellow to orange-red. With regards to human

health, lutein helps maintain healthy eyes, heart and metabolism (Aston *et al*., 2006). Although not considered essential, without lutein and carotenoids in general, impaired vision and health may occur. Nutrition professionals recommend more than 1 -3 mg per day of lutein and its isomer zeaxanthin. The lutein content in three different reports on avocado tissue was inconsistent. The USDA database (2016) reports 271µg of lutein and zeaxanthin in 100g of fresh avocado. Hass avocados grown in California and harvested in September were found to have 842µg lutein, 1197µg neoxanthin, and 475µg violaxanthin in 100g of avocado tissue, showing xanthophylls were predominate in the Hass avocado (Lu *et al*., 2009). Hass avocados grown in Whangarei, New Zealand had 180µg lutein in 100g of avocado flesh, violaxanthin and zeaxanthin had less than 10µg per 100g flesh (Ashton *et al*., 2006). The discrepancies in carotenoid content may be attributed to the tissue being sampled, as indicated by the difference in color, the cultivar being tested, and the extraction procedure (Chen *et al*., 2009). The use of avocado oil addresses the issue of the location of tissue sampling because in the process of extracting oil, all the tissue (from the dark green portion to the yellow portion) is used. This could be the reason why extracted avocado oil has the highest concentrations of carotenoids compared to the raw flesh with 2300g carotenoid per 100 grams of oil (Ashton *et al*., 2006).

Other antioxidants in 100g of raw avocado include 10mg of Vitamin C, 2.07mg of α-tocopherol, and 0.33mg of γ-tocopherol (USDA, 2016). The α-tocopherol content value varied in the literature. The Fortuna cultivar grown in Pelotas, Brazil was found to have 2.8mg of α -tocopherol per 100g of avocado tissue (dos Santos *et al*., 2014). Hass avocados grown in Mexico had 3.228mg α-tocopherol per 100g of tissue (Peraza-Magallanes *et al*., 2017). While Hass avocados grown in California had 1.63 – 2.76mg of α -tocopherol per 100g of flesh (Lu *et al*., 2009). The difference in α-tocopherol values was attributed to genetic background enhanced by maturity stage, environmental and geographical conditions, harvest season, postharvest management, and agronomic techniques (Peraza-Magallanes *et al*., 2017).

1.7 Health Benefits

Avocados have generated great interest in recent years due to their promising potential as a natural functional food. Unlike functional foods, natural functional foods have inherent health-promoting

effects related to their chemical composition, which does not need to be modified to enhance their effects. The avocado has been classified as a functional food by the Academy of Nutrition and Dietetics (1999) for over a decade due to their high nutritional value and beneficial effects on human health. Health benefits from the consumption of the fruit encompass preventing diet related diseases as well as managing and preventing these diseases. Such health diseases include cardiovascular disease, diabetes, high adiposity accumulation, chronic low-grade systemic inflammation, dyslipidemia, hypertension and digestive cancers.

Nutrition data from the National Health and Nutrition Examination Survey (NHANES) revealed those who consumed avocados (non-specific variety) had lower body weight, BMI, and waist circumference (Fulgoni *et al*., 2013). Researchers used cross-sectional epidemiological data collected from 17,567 American adults (≥19 yrs) from 2001 – 2008 to investigate the association between avocado intake and diet quality, energy and nutrient intakes, body weight and metabolic syndrome risk factors. Metabolic syndrome is the umbrella term for a group of health conditions that synergistically increase the risk of heart disease, stroke and diabetes (Fulgoni *et al*., 2013). Dietary habits were collected from a 24hr recall from participants NHANES using the Automated Multiple Pass Method (AMPM). Physiological information was collected from the participants at mobile NHANES centers. Diets including avocados were compared to diets without avocados. The dietary data revealed there was no discrepancy in calorie and sodium intakes among those who ate avocados and did not. Avocado consumers did have higher intakes of total fat, unsaturated fats, dietary fiber, vitamins E and K, magnesium and potassium. They also had lower carbohydrate intake. The physiological data showed avocado consumers had lower body weight, BMI, and a smaller waist circumference. They also had a healthier lipid profile with higher HLD-C levels compared to non-consumers. The risk for metabolic syndrome was 50% lower in consumers than non-consumers. The researchers concluded avocados should be a part of people's diet due to their health benefits, especially those who have an increased risk for metabolic diseases (Fulgoni *et al*., 2013). Findings using the NHANES database are estimates and can be generalized to the population at large.

In a well-controlled human study the effect of Hass avocado consumption on cardiovascular disease risk factors was assessed (Wang *et al*., 2015). The researchers wanted to specifically evaluate

the nutrients and bioactive compounds besides the monounsaturated fatty acids present in the avocados. In a randomized, crossover, controlled feeding trial of overweight to obese people (n=45) with LDL-C in the 25th – 90th percentile, three diets were consumed. Each diet was eaten for 5 weeks and were separated by 2 week breaks where subjects consumed the Average American diet. The American diet was also consumed before the first arm of the study. The study participants were advised to maintain a normal level of physical activity throughout the study. The three diets were isocaloric depending on the participants' energy needs, and were matched for saturated fatty acids. The low fat diet consisted of 24% fat, 59% carbohydrate, 16%protein and 25% fiber. The moderate fat diet consisted of 34% fat, 49% carbohydrate, 16% protein, and 26% fiber. Most of the fat sources in the moderate fat diet were from high oleic acid oils such as mayonnaise, canola oil, sunflower oil and margarine. The avocado diet consisted of one fresh Hass avocado a day which contributed to the overall 34% fat in the diet. The other fat sources were dairy products. The avocado diet also consisted of 49% carbohydrate, 16% protein, and 35% fiber. Biochemical data was collected at the end of the first American diet phase and after each experimental diet. The fasting serum samples showed the avocado diet significantly decreased LDL-C compared to the other two diets. Furthermore, the Hass avocado diet was the only diet that significantly reduced LDL cholesterol (Wang *et al*., 2015). The difference in results between the moderate fat diet and avocado diet suggests other nutrients and bioactive compounds (e.g. phytosterols, dietary fiber) beyond fatty acids may be responsible for the health benefits of avocados. The research supports the daily inclusion of a nutrient-dense avocado will contribute to a more favorable lipid/lipoprotein profile and smaller LDL size (Wang *et al*., 2015).

It is generally agreed that a diet rich in fruits and vegetables is associated with a reduced risk of common forms of cancer. Phytochemicals possess chemo-preventive properties involved in suppressing, blocking or reversing the process of carcinogenesis. Such phytochemicals include carotenoids, flavonoids, and vitamins (Lu *et al*., 2005). Based on the yellow-green color of avocados, researchers examined the carotenoid content and other bioactive substances in lipid extracts from the Hass avocados for an anti-proliferative effect. The research demonstrated the growth inhibition of androgen dependent (LNCaP) and non-androgen dependent cancer cells by an acetone extract from the Hass cultivar. Lu *et al*. (2005) attributed the observed bioactivity of the fruit to the high contents of lutein and vitamin E (α-

tocopherol and gamma-tocopherol) in the avocado extracts. The effects of the carotenoids were enhanced by the presence of fatty acids, particularly the monounsaturated fats. The unsaturated fatty acids helped to increase the bioavailability of bioactive carotenoids into the bloodstream. Although the data was collected *in vitro*, the anti-cancer potential of Hass avocados was preliminarily established. Clinical trials expounding on this early evidence are crucial in demonstrating the health benefits of avocados. In general, it can be stated that the carotenoids and other lipophilic antioxidant compounds present in avocados may impart the benefits seen in the fruit's inclusion into a healthy diet (Lu *et al*., 2005).

Despite the high lipid content in avocados, the fruit helps to control weight with potential to aid in weight loss. An intervention study investigated the effects of avocado inclusion in an energy-restricted diet on weight loss, serum lipids, fibrinogen, and vascular function and found health benefits of the fruit (Pieterse *et al*., 2005). In a controlled parallel study design, 61 participants were randomly assigned to one of two groups for 6 weeks. The subjects were either overweight or obese. The experimental group had 200g of avocado (non-specific variety) incorporated into their daily diet. The control group had 30g of mixed dietary fats (e.g. margarine, mayonnaise, oil) in their diet instead of avocado. Both diets were isoenergetic and energy-restrictive to induce weight loss. At the conclusion of the study, researchers found eating the fat-dense avocado did not compromise weight loss. Avocado intake also did not negatively affect serum lipid concentrations, fibrinogen, blood pressure, and arterial compliance. These results were based on anthropometric measurements of all subjects (Pieterse *et al*., 2005).

The antioxidant and anti-inflammatory properties of avocados aid in the digestion and absorption of animal meat products by reducing pro-inflammatory and vasoconstrictory effects (Li *et al*., 2013). Researchers examined the effect of Hass avocados coupled with hamburger meat on postprandial vasodilation and inflammatory response to the meal (Li *et al*., 2013). Eleven healthy male participants were fed a 250g beef patty with 25g of fat and the same beef patty with 68g of Hass avocado. This was done on two separate occasions separated by a week in a randomized crossover study design (Li *et al*., 2013). Blood and urine samples were collected every hour for 6 hours following consumption. Peripheral arterial tonometry was done before eating and 2hrs after eating. The results revealed avocados helped

attenuate the postprandial vasoconstrictive effect of the high fat beef patty (Li *et al*., 2013). Furthermore, postprandial plasma interleukin 6 levels were lower after consuming the avocado meal compared to the regular meal. This suggests the fruit has a cardioprotective mechanism which is significant considering chronic inflammation may have a role in atherosclerosis and cardiovascular disease development (Li *et al*., 2013). Health professionals may recommend the dietary inclusion of avocados due to their beneficial anti-inflammatory and vascular health effects in a high protein and fat meal.

Separate clinical studies have found avocado consumption improved lipid profiles in subjects with Type 2 diabetes and in subjects with mild hypercholesterolemia (Lerman-Garber *et al*., 1994; Lopez *et al*., 1996). In a randomized controlled study, 67 adult participants ate an avocado-enriched diet or a non-avocado diet (Lopez *et al*., 1996). Of the 67 subjects, 30 were healthy while the remaining 37 had mild hypercholesterolemia. Those who consumed the avocado diet were 30 adults with high cholesterol and 15 healthy adults. The avocado diet had 300g of Hass avocado while the non-avocado diet had 300g of fat from other sources. The diets were isocaloric and eaten for seven days. Among the 30 mildly hypercholesterolemia patients consuming the avocado diet, there were decreases in the levels of total serum cholesterol, LDL and triglycerides, while HDL levels increased. No significant changes were seen in subjects who consumed the non-avocado diet (Lopez *et al*., 1996). The research supports the hypothesis that high lipid avocado fruit can improve lipid profiles in people with and without high cholesterol and triglyceride levels.

Research by Lerman-Garber *et al*. (1994) found similar lipid profile improvements from consuming avocados. In a randomized crossover study, complex carbohydrates were replaced with avocados and olive oil (rich sources of unsaturated fats) in the diets of 12 females with Type 2 diabetes. The participants were randomly assigned to two study diets (high fat diet or high carb diet) with each diet being consumed for 4 weeks with a washout period separating them. Blood samples were taken before and after each dietary period. Results showed the high fat diet was associated with a decrease in plasma triglycerides. The researchers concluded a diet rich in unsaturated fatty acids helped Type 2 diabetics manage their lipid profile favorably (Lerman-Garber *et al*., 1994).

1.8 Conclusion

The support for avocados being a healthy dietary choice has been increasing since the 1990s. The Hass cultivar is one of the most common avocados traded and consumed annually, being a relatively easy crop to cultivate and one with a preferred taste among buyers. However, there are many avocado cultivars grown and sold in the Hass-centric market which complicates the applicability of the nutritional and health information on avocados since these both are based on the Hass cultivar. The USDA nutrient database, considered the "go-to" for all nutritional information on foods, does not have specific nutrient content information for the many varieties seen in the US market place. Rather the database is America-centric, displaying information on avocados grown in California or Florida. This is not relevant to the US public as the majority of the avocados imported into the US come from abroad, mostly from Mexico. There is robust support for the variation in nutrient content among cultivars grown in different regions, with the slogan "not all are the same" being easily applied. The avocado industry in America has potential to grow (i.e. increase yield and supply the consumer demand) given needed help in understanding the growing needs of the tree. Such needs include suitable temperatures, rainfall/water, and energy needed to prevent stressful conditions. Using the agriculture technologies of today, the US can be more sustainable in their avocado consumption.

Hawaii is crucial in developing a sustainable avocado industry due to the climate and geography of the islands being naturally conducive to the optimal growth of the tree. Avocados have been thriving in Hawaii with very little agricultural management for the last 200 years (Chan-Halbrendt *et al*., 2007). As a result, hundreds of cultivars are growing, with many of them being sold for human consumption and competing with the Hass avocado for share in the local market. If efforts to increase Hawaii avocado production come to fruition, there is a need to expand the national nutritional database to include Hawaii grown cultivars. Currently such inclusion is non-existent; in fact, nutrient information on Hawaiian avocados is not accessible to the public. This significantly limits the marketing of locally grown avocados and the expansion of the industry in the islands. One purpose of the research in this thesis was to identify the nutrient content of Hawaii grown avocados, especially the mineral profile and carotenoid profile, both of which had never been done. This data can be used to support the wide range of health benefits of locally grown avocados leading to improved marketing strategies to increase their purchase. Such information is valuable to industry stakeholders. Farmers want to be assured their produce will be

purchased at a price that will generate revenue (Barbara *et al*., 2008). The data from this research will help to determine the most suitable cultivars for which to devote precious resources from a nutritive and commercial perspective. Committing to the appropriate avocado varieties ensures fruits that are most advantageous for health. The other purpose of this research was to provide an international comparative study. In quantifying and comparing the nutrition of Hawaii and African (i.e. Cameroonian) avocados we showed the variation in nutrient content. This supports the description of each variety being inherently different and therefore warranting individual nutrient profiles. Not all avocados are the same was the overall agreement reached (Love, 2009).

Chapter 2
Materials and Methods

2.1 *Sample Collection*

Hawaii grown avocados used for this study were harvested in February 2016 from farms in South Kona, Hawaii. The elevation of the farms ranged from 300 – 580m. The six cultivars were Behsore, Linda, Murashige, Nishikawa, Ohata and Serpa. There were four Beshore fruits, four Linda fruits, two Murashige fruits, seven Nishikawa fruits, two Ohata fruits, and four Serpa fruits. The fruits were unripe upon pick-up and within the same day, air flown to University of Hawaii at Manoa. The stage of ripeness was determined by firmness and color of the fruit. Unripe avocados were green and no indenture remained on the skin surface when pressed. Once at the UH Manoa campus, fruits ripened at room temperature between five to seven days after arrival. Avocados were determined to be ripe when a slight indenture remained on the skin surface when pressed.

Cameroon grown avocados used for this study were harvested in June 2016 from farms in Foumbot, Cameroon in Central Africa. The farms were under the care and direction of the Institute of Agricultural Research for Development, a government-operated research organization under the Cameroon Ministry of Scientific Research and Innovation and the Ministry of Economy and Finances. Cameroon avocados were grown at an elevation of 1000m. The four Cameroon grown varieties analyzed were BoothVIII, Fuer Florida, Peterson and Pollock. There were nine BoothVIII fruits, eleven Fuer Florida fruits, eleven Peterson fruits, and four Pollock fruits. Avocados were harvested unripe based on color and firmness and transported to Yaoundé, Cameroon to the Institute of Medical Research and Medicinal Plant Studies (IMPM). Fruits were brought at IMPM unripe and ripened in five days at room temperature. Avocados were determined to be ripe when an indenture was left on the skin surface when pressed.

2.2 *Sample Preparation and Processing*

Hawaii grown avocados were prepared and processed at the University of Hawaii Manoa. Each ripe avocado fruit was weighed in its entirety for a “whole” weight. The ripe fruit was then cut vertically in half and the seed and peel were removed. For this nutrient analysis, the seed and peel were considered inedible and weighed for a “refuse” weight. The mesocarp of the avocado fruit was considered edible.

The edible portion was cut horizontally into slices that were 2.5 – 5cm long and 1.3cm thick. The slices were then weighed for a “fresh wet” weight. The mesocarp was frozen at -15°C in preparation for lyophilization. The frozen mesocarp was freeze dried at -54°C at 0.033mBar for 48 hours (FreeZone Freeze Dry Systems, LabConco, Kansas City, MO). Immediately after lyophilization, the samples were weighed for a “dry” weight and were stored in plastic containers in desiccators until further analysis.

Cameroon grown avocados were prepared and processed at IMPM in Yaoundé, Cameroon. All the fruits for each variety were pooled and processed and prepped together for analysis. Processing and prepping happened on the same day for all cultivars which was five days after their arrival at the IMPM laboratory. All the fruits for each variety were weighed together for a “whole” weight, then cut to remove the seed and peel. The seed and peel were weighed for a “refuge” weight. The mesocarp was cut into slices and weighed for a “fresh wet” weight. The fruits were frozen at -15°C prior to lyophilization. Samples were freeze dried for 48 hours (Christ Beta 1 – 8 series, BioBlock Scientific, mention name of city, state and country if not from US). Samples were immediately weighed after the drying cycle to capture the “dry” weight. After weighing, the mesocarp for each cultivar was pooled in containers and stored in desiccators until further analysis.

2.3 *Moisture Content*

The moisture content of the ten avocado cultivars was determined according to the AOAC method 930.15 (2006). The analysis of both Hawaiian and Cameroonian cultivars was done at IMPM in Yaoundé, Cameroon. The freeze dried avocado samples were ground into a powder using a clean mortar and pestle. The mortar and pestle were cleaned after each cultivar. Samples were weighed into pre-weighed containers. Containers with samples were heated to 100°C for 6 hours. After air drying, the containers were weighed immediately.

Air drying was done to freeze dried samples. The process of freeze drying does not remove the residual moisture content. Moisture content was calculated based on the weight difference between the fresh wet weight and freeze dried weight as well as the weight difference between the freeze dried weight

and air dried weight. These differences were then summed and divided by the fresh wet weight. The following calculation was done for each cultivar to determine the moisture content:

$$Moisture\ Content = \frac{(Fresh\ wet\ wt - Freeze\ dried\ wt) + (Fr\square eze\ dried\ wt - Air\ dried\ wt)}{Fresh\ wet\ wt}$$

2.4 *Crude Protein Content*

The crude protein content of the ten cultivars was determined according to AOAC 2006. The nutrient analysis of both Hawaiian and Cameroonian cultivars was done at IMPM in Yaoundé, Cameroon and were ran in duplicates. The Kjeldahl digestion method was used for protein content analysis (method 976.05). The lyophilized avocado mesocarp was ground into a powder using a clean mortar and pestle. The mortar and pestle were cleaned after each cultivar. The ground samples were weighed and placed onto pre-weighed filter paper that was then folded. The samples were placed in separate digestion tubes to which 25mL of concentrated sulfuric acid and catalyst tablets were added to each. Contents in the tubes incubated for an hour while on the tube rack. After incubation, the rack was placed in a preheated block (420°C). The samples were digested for 3 hours or until the tube contents became clear. After digestion, the tubes were cooled down and 100mL of distilled water was added to each tube.

The digestion tubes were attached to the distillation unit. In the distillation flask, 80mL of 40% sodium hydroxide and zinc pieces were added. Large titration flasks were filled with 100mL of boric acid, 300mL of distilled water, and 3 – 4 drops of methyl red indicator. Titration flasks were placed under burners and the distillation flask was corked and heated. The distillation flask was boiled until 300mL of solution was distilled into the titration flasks. The solution in the titration flasks was then titrated with sulfuric acid (0.255N). Titration was complete when the solution in the flasks changed colors from blue to green. The amount of sulfuric acid used to titrate was recorded. The following calculations were done to find the crude protein content:

$$Crude\ protein\ (\frac{g}{kg}) = \frac{(14\ x\ molarity\ of\ the\ acid)(titer\ value - blank)}{sample\ weight}\ \text{and}\ nitrogen\ x\ 6.25$$

2.5 *Insoluble Fiber*

The insoluble fiber content of the avocado cultivars was determined according to AOAC method 973.18 (2006). The analysis of both Hawaiian and Cameroonian cultivars analysis was done at IMPM in Yaoundé, Cameroon. The freeze dried avocado samples were ground into a powder using a clean mortar and pestle. The mortar and pestle were cleaned after each cultivar. The samples were weighed, placed in separate beakers, and mixed with sulfuric acid (0.255N). This mixture was boiled and reflux for 30 minutes after which the residue was filtered. The filtrate was mixed with sodium hydroxide (0.313N), boiled and refluxed for another 30 minutes and filtered. The residue was washed three times with hot distilled water then acetone washed twice. The washed residue was then dried at 105°C for 8 hours and then was weighed. After weighing, the residue was incinerated for 3 hours at 550°C. The remaining ash product was then weighed.

Insoluble fiber was determined based on weight differences between residue and ash and took into consideration total lipid content. The following calculation was done to determine the crude fiber content:

$$\text{Insoluble Fiber} = \frac{(Residue\ weight - Ash\ weight)\ (100 - total\ lipid)}{Sample\ weight}$$

2.6 *Mineral Content*

The total mineral content of the ten cultivars was determined according to AOAC 1980. The mineral content measurement of both Hawaiian and Cameroonian cultivars was done at IMPM in Yaoundé, Cameroon and run in duplicates. The freeze dried avocado samples were ground into a powder using a clean mortar and pestle. The mortar and pestle were cleaned after grinding each cultivar to avoid any contamination. Samples were weighed into pre-weighed containers. Containers with samples were heated to 650°C for 16 hours. After incineration, the containers were cooled and weighed. The difference in weights was used to determine the total mineral content. The following calculation was done for each cultivar to determine the crude mineral content:

$$Total\ Mineral\ Content = \frac{(Sample+Container) - (Ash+Container)}{Sample\ weight}$$

2.7 *Crude Lipid Content*

The crude lipid content of the ten cultivars was determined according to AOAC 1980. The nutrient analysis of both Hawaiian and Cameroonian cultivars was done at IMPM in Yaoundé, Cameroon and were ran in duplicates. The crude fat was extracted from the samples using petroleum ether as a solvent and a Soxhlet apparatus (Medoua et al., 2009). The freeze dried avocado samples were ground into a powder using a clean mortar and pestle. The mortar and pestle were cleaned in between grinding each cultivar. Between 0.3 – 0.7 grams of sample was weighed out on a pre-weighed filter paper, with the exact weight being recorded. The sample was placed in the Soxhlet apparatus and 50 mL of solvent was poured into the round bottom collection flask. Extraction was done at 100°C for 7 hours or until solvent drained clear from the sample. The filter paper with sample residue was dried at room temperature for an hour and weighed. The solvent was evaporated using a rotary evaporator and the oil was collected in tubes for fatty acid analysis. The crude lipid content was determined from the weight difference between sample and residue according to the following equation:

$$Crude\ Lipid = \frac{(Sample+Filter\ paper)-(Residue+Filter\ paper)}{Sample\ weight}$$

2.8 *Fatty Acid Profile*

The fatty acid profile of the ten avocado cultivars was determined using liquid chromatography mass spectrometry (LCMS) following the technique described by Li and Franke (2011). The analysis of both Hawaiian and Cameroonian cultivars was done at the University of Hawaii Cancer Center in Honolulu, HI and all samples were run in triplicate. The fatty acid standards (Cayman Chemicals, Ann Arbor, MI) were stearic acid (SA), oleic acid (OA), linoleic acid (LA), arachidonic acid (ARA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), palmitic acid (PA), eicosadienoic acid (EDA), docosapentaenoic (DPA), and palmitoleic acid (PLA). Myristic fatty acid (MA) were

purchased from Sigma-Aldrich (St. Louis, MO). The ten avocado fat samples were extracted from freeze dried ground samples via Soxhlet apparatus. The sample oils and fatty acid standards were hydrolyzed and derivatized in preparation for LCMS analysis.

Hydrolysis of the avocado fatty acids was done by weighing 5-10mg of each sample and adding 20uL of internal standard to each. This was followed by adding 50uL of butylated hydroxytoluene antioxidant (BHT) to each sample then adding 850uL of methanol/dichloromethane (1/1, v/v). This was followed by adding 150uL of 40% potassium hydroxide (KOH) and letting mixture incubate for 30min at 60°C. After incubation, 700uL of phosphate-buffered saline (PBS) was added. The pH was lowered to 1-2 by adding HCl, after which the mixture was washed twice with diethyl/hexane (1/1, v/v). The organic layer of the mixture, which contained the free fatty acids, was aliquoted and dried under nitrogen gas.

The hydrolyzed free fatty acids were then derivatized. First, 150uL of oxalyl chloride (2M in DCM) was added to each sample then vortexed. The mixture was then heated for 5min at 65°C and dried under nitrogen gas. To the dried mixture, 100uL of 3-aminomethyl pyridine (3PA) (2% v/v, 3PA/acetonitrile) was added along with 500uL of ethanol. To the standards, 100uL of ethanol was added. Samples and standards were pipetted into separate HPLC vials. From the vials, 10uL of the mixture was injected into the LCMS (HTC Pal autosampler, Leap Technologies, connected to Accela ultra-HPLC system with Exactive orbitrap mass spectrometer, Thermo Electron, add city and state name).

The column used was Agilent Zorbax SC-C18 column (3.0x5mm, 1.8um, Agilent). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 500uL/min. For the separation of the fatty acid-picolylamine derivatives, the linear gradient used was A/B (v/v): 0-7min 35/65, 7.1-11min 10/90, and 11.1-15min 35/65 (Li and Franke, 2011).

The mass detection was done following a positive-ion full scan mode after electrospray ionization (ESI). The settings of the mass spectrometer were: 4.5kV spray voltage; 250°C capillary temperature; 250ms for the maximum injection time; and 100 – 650 scan rage. Nitrogen was used for both the sheath gas (pressure 30units) and auxiliary gas (pressure 10units). The in – source collision induced dissociation energy (CID) was set at 5eV. This was determined to dissociate the dimers or sodium adducts. The

automatic gain control (AGC) was set at balanced. The software Xcalibur (Thermo Fisher Scientific, Waltham, MA) was used for the data acquisition and analysis. Detection of the analyte was set within 10 ppm of the calculated mass.

2.9 *Mineral Profile*

The mineral analysis was done at the University of Missouri Agricultural Experiment Station Chemical Laboratories (ESCL, Colombia, MO). The analysis was done according to AOAC 985.01 and AOAC 990.08. The measurements of the specific minerals were profiled using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP – OES). Avocado samples were freeze dried and ground at the University of Hawaii Manoa campus and mailed to ESCL. At ESCL, the samples were dried and ground then solubilized into a liquid. The analyses were done on an "as is" basis. Results were presented in a Microsoft excel file and in either grams per 100 grams of sample (W/W%) or in parts per million or milligram per kilogram of sample (ppm).

2.10 *Lutein, Neoxanthin, Violaxanthin Profile*

The carotenoid profile of the ten avocados was analyzed at the University of Hawaii, Manoa campus. Lutein, neoxanthin, and violaxanthin were determined using the techniques of reverse-phase high-performance liquid chromatography (RP-HPLC) (Carlos-Hilario et al., 2015). In this system, the liquid eluant moves through the column. The stationary phase adheres extremely nonpolar compounds, while lesser nonpolar hydrophobic molecules are eluted. Compounds move off the column based on their degree of hydrophobic characteristic. This analysis was done in triplicates. Solvent A (1/9/0.1, v/v/v, deionized water/acetonitrile/triethlamine) and was used against Solvent B (ethyl acetate). Ethyl acetate binds to hydrophobic organic compounds but not to lipids. The freeze dried, ground samples were re-ground in liquid nitrogen and lyophilized for 48 hours. Immediately after freeze drying, samples were weighed and put into labeled tubes along with Solvent A (target ratio: 5mg of sample/1mL of Solvent A).

Tubes were sonicated (30mins) and centrifuged (13K rmp, 10mins). The aqueous layer was then pipetted out into another tube, making sure no solid material was taken up, and sonicated (30min) and centrifuged (10min) again. From this, 200uL of sample was pipetted into HPLC vials. Sample was injected into the HPLC machine at 100uL quantity (Waters 2695 automated HPLC system with Waters 966 Photodiode Array Detection(PDA), scanning 210 – 600 nm).

The column used was a Phenomenox C18 Kinetex HPLC Column at a flow rate of 0.8mL with a linear 8%/min gradient of Solvent A against Solvent B (Carlos-Hilario et al., 2015). In between sample injection, there was a 3min re-equilibration. The resulting chromatographic profiles were extracted at 430nm. The target peak area was integrated using Waters Empower Pro software.

2.11 *Statistical Analysis*

The results from each analysis were used to calculate the mean and standard error of the mean for cultivar. The significant differences between the means of each cultivar were calculated by a one-way analysis of variance (ANOVA). These differences were held at a 95% confidence interval ($P<0.05$) using the Tukey's range test. These analyses were done on JMP Pro 13.

Chapter 3
Results

3.1 *Proximate Nutrient Analysis*

The proximate nutrient analysis of the ten unique avocado cultivars are presented in Table 1. The moisture content among all ten cultivars ranged from 58.96g/100g fresh wt. in the Nishikawa cultivar to 79.73 g/100g fresh wt. in the Ohata cultivar. Both Nishikawa and Ohata cultivars were grown in South Kona, Hawaii. Hawaii grown avocado cultivar Linda had a moisture content of 76.38g/100g fresh wt. and Murashige had 75.36g/100g fresh wt. Moisture content in Cameroon grown avocados was as followed: Pollock had 78.06 g/100g fresh wt., Peterson had 69.42g/100g fresh wt., BoothVIII had 66.90 g/100g fresh wt. and Fuer Florida had 63.84g/100g fresh wt. Moisture content was done in one single run resulting in one data point for each cultivar.

The insoluble fiber content among all the ten avocado cultivars ranged from 2.30g/100g fresh wt. in the Peterson cultivar to 0.78g/100g fresh wt. in the Linda cultivar. The insoluble fiber content in the Cameroon grown avocados were as follows: BoothVIII had 1.75g/100grams fresh wt., Fuer Florida had 1.53g/100g fresh wt. and Pollock had 1.37g/100g fresh wt. Hawaii grown avocado cultivars had the following insoluble fiber content: Nishikawa (1.51g/100grams fresh wt.), Murashige (1.16g/100g fresh wt.), Beshore (0.92g.100g fresh wt.), Serpa (0.93g/100g fresh wt.), Linda (0.78g/100g fresh wt.) and Ohata (0.81g/100g fresh wt.). Insoluble fiber content was done in one single run resulting in one data point for each cultivar.

The total protein content among all ten avocado cultivars ranged from 0.79g/100g fresh wt. in the Linda cultivar to 2.56g/100g fresh wt. in the BoothVIII cultivar. The Cameroon grown cultivars BoothVIII and Peterson had large total percent protein content at 2.57g/100g fresh wt. and 2.40g/100g fresh wt. respectively. The protein content in BoothVIII was significantly larger compared to Linda, Serpa, Nishikawa, Murashige, Ohata, Beshore, and Pollock ($p<0.0001$). The Hawaii grown Linda cultivar had protein content of 0.79g/100g fresh wt., which was less than Peterson and Fuer Florida ($p<0.0001$). Serpa (0.97g/100g fresh wt.) and Nishikawa (1.14g/100g fresh wt.) had a non-significant difference in protein content ($p=0.8901$). Protein content in Linda, Serpa and Nishikawa were also not significantly different ($p>0.05$). Protein content was not significantly different among the Hawaiian avocados Beshore,

Ohata, Murashige, Nishikawa and Serpa ($p>0.05$). Among the Cameroon grown avocados, Peterson and Fuer Florida had a higher protein content compared to Pollock ($p=0.0002$, $p=0.0064$ respectively).

The total lipid content among all the ten avocado cultivars ranged from 35.33g/100g fresh wt. in the Nishikawa cultivar to 14.15g/100g fresh wt. in the Pollock cultivar. The Nishikawa cultivar had a significantly higher lipid content compared to Pollock, Ohata, Peterson, Murashige and Linda ($p<0.0001$). The lipid content in the Pollock cultivar was significantly lower than Fuer Florida ($p<0.0001$) and BoothVIII, Serpa and Beshore ($p=0.0002$). Pollock did not significantly differ from Ohata and Peterson ($p=0.4660$, $p=0.1801$ respectively). Linda did not have a significantly different lipid content compared to BoothVIII, Serpa, and Behsore ($p=0.0503$, $p=0.0518$, $p=0.0557$ respectively). Among the Cameroon grown cultivars, lipid content was not significantly different among Fuer Florida and BoothVIII ($p=0.2330$); it was also not significantly different between Peterson and Pollock ($p=0.1801$).

The total mineral contents among all the ten avocado cultivars ranged from 1.64g/100g fresh wt. in the BoothVIII cultivar to 0.18g/100g fresh wt. in the Serpa cultivar. Total mineral content among all four Cameroon avocados was not statistically different ($P>0.05$); BoothVIII had 0.18g/100 g fresh wt., Peterson had 1.39g/100g fresh wt., Fuer Florida had 1.36g/100 g fresh wt., and Pollock had 0.82g/100g fresh wt. Total mineral contents among all six Hawaii avocados were not statistically different ($P>0.05$). Cameroonian BoothVIII has a larger total mineral content compared to Hawaiian cultivars Serpa ($p=0.0026$), Murashige ($p=0.0031$), Nishikawa ($p=0.0072$), Linda ($p=0.0126$) and Ohata ($p=0.0281$).

Table 1.1. Proximate nutrient analysis of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Beshore Mean	BoothVIII Mean	FuerFlorida Mean	Linda Mean	Murashige Mean	USDA Reference	P-value
Moisture %wt	70.44	66.90	63.84	76.38	75.36	73.23	
Fat %wt	26.0±1.0^{BC}	26.0±1.0^{BC}	30.0±1.0^{AB}	21.0±1.0^{CD}	20.0±0.2^{D}	14.66	<0.0001
Protein %wt	1.4±0.1^{C}	2.6±0.1^{A}	2.1±0.3^{B}	1.0±0.1^{D}	1.3±0.1^{C}	2.00	<0.0001
Ash %wt	1.0±0.2^{ABC}	2.0±0.4^{A}	1.4±0.3^{AB}	1.0±0.2^{BC}	0.2±0.2^{C}	1.58	0.0007
Insoluble Fiber %wt	0.92	1.75	1.53	0.78	1.16		

Different letters in the same row indicate statistically significant differences (Tukey's Test, $p<0.05$). Mean±SD, standard deviation (n=2).

Table 1.2. Proximate nutrient analysis of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Nishikawa Mean	Ohata Mean	Peterson Mean	Pollock Mean	Serpa Mean	USDA Reference	P-value
Moisture %wt	58.96	79.73	69.42	78.06	69.89	73.23	
Fat %wt	35.0±3.0^{A}	17.0±1.0^{DE}	18.0±2.0^{DE}	14.0±1.0^{E}	26.0±0.4^{BC}	14.66	<.0001
Protein %wt	1.1±0.01^{CD}	1.3±0.1^{C}	2.4±0.1^{AB}	1.4±0.1^{C}	1.0±0.03^{CD}	2.00	<.0001
Ash %wt	0.4±0.4^{C}	1.0±0.1^{BC}	1.4±0.3^{AB}	0.8±0.01^{ABC}	0.2±0.02^{C}	1.58	0.0007
Insoluble Fiber %wt	1.51	0.81	2.30	1.37	0.93		

Different letters in the same row indicate statistically significant differences (Tukey's Test, $p<0.05$). Mean±SD, standard deviation (n=2).

3.2 *Mineral Profile*

The mineral profiles of avocado cultivars are present in Table 2. Potassium content was significantly higher ($P<0.0001$) in the Cameroon cultivar BoothVIII (0.82g/100g fresh wt.) compared to all other cultivars. The Peterson cultivar had more potassium than Murashige, Linda, Pollock, Ohata, Nishikawa, and Serpa ($p<0.0001$). Fuer Florida (0.70g/100g fresh wt.) and Beshore (0.68g/100g fresh wt.) did not have significantly different potassium content ($p=0.3242$). Ohata (0.41g/100g fresh wt.) and Pollock (0.41g/100g fresh wt.) also did not have significantly different potassium content ($p=1.0$). The Hawaii grown cultivar Murashige (0.31g/100g fresh wt.) had the lowest potassium content compared to all other cultivars ($p<0.0001$).

Calcium content was highest in Linda (0.019g/100g fresh wt.), Peterson (0.018g/100g fresh wt.) and Fuer Florida (0.018g/100g fresh wt.) compared to the other cultivars ($p<0.0001$). Hawaii grown avocados, Murashige (0.008g/100g fresh wt.), Serpa (0.008g/100g fresh wt.) and Ohata (0.006g/100g fresh wt.) had non-significantly different calcium content ($p>0.08$). BoothVIII and Pollock had significantly higher calcium contents than Ohata ($p<0.0001$). There was no significant difference in calcium content between BoothVIII and Pollock ($p=0.9931$). Beshore, Nishikawa, Murashige and Serpa had non-significant differences in calcium content ($p>0.08$).

Cameroonian avocados Fuer Florida and Peterson had non-significantly different magnesium contents ($p=1.0$). These two cultivars, however, were significantly higher in magnesium compared to all the other cultivars ($p<0.0001$). Magnesium content in the Hawaiian Nishikawa cultivar was significantly different than all the other cultivars ($p<0.0001$). The Hawaii grown Ohata cultivar had the lowest magnesium content (0.017g/100g fresh wt.) among all avocados ($p<0.0001$). The magnesium content in Murashige, Pollock, BoothVIII, Serpa, Beshore and Linda were not significantly different ($p>0.3$).

The sodium content in the Hawaiian avocados Ohata (35.8mg/kg fresh wt.) and Murashige (33.77mg/kg fresh wt.) was significantly higher than in the other cultivars ($p<0.0001$). Cameroon avocados Pollock, BoothVIII and Fuer Florida had significantly lower sodium content compared to Linda, Serpa, Nishikawa, and Beshore ($p<0.0001$). Sodium content as not significantly different among Pollock,

BoothVIII and Fuer Florida ($p>0.3$). Sodium content was also not significantly different among Serpa, Nishikawa and Beshore ($p>0.2$).

Cameroon avocados Peterson (12.49mg/kg fresh wt.), BoothVIII (12.03mg/kg fresh wt.) and Fuer Florida (11.74mg/kg fresh wt.) had the highest iron content compared to the other cultivars ($p<0.0001$). The aforementioned avocados had non-significant differences in iron content ($p>0.4$). Hawaiian cultivars Ohata, Murashige and Linda had the significantly lower iron content compared to the other cultivars ($p<0.0001$). The iron content among Ohata, Murashige and Linda was not significantly different ($p>0.9$). Iron content was also not significantly different among cultivars Pollock, Beshore, Serpa and Nishikawa ($p>0.4$).

The manganese content in the Hawaiian cultivar Nishikawa was 2.0mg/kg fresh wt., which was the largest among all the cultivars ($p<0.0001$). Cameroon cultivar Pollock had a lower manganese content compared to BoothVIII, Murashige, Fuer Florida, Peterson, Linda, Beshore and Serpa ($p<0.0001$). Manganese content did not significantly differ among Fuer Florida, Murashige, BoothVIII, and Ohata ($p>0.1$). Serpa and Beshore had non-significantly different manganese content ($p=0.6928$), but these two had significantly more manganese than Linda, Peterson, and Fuer Florida ($p<0.0001$).

The Cameroon avocado cultivar BoothVIII had a zinc content of 9.53mg/kg fresh wt. BoothVIII had a significantly larger zinc content compared to the other cultivars ($p<0.0001$). Peterson had a zinc content larger than Fuer Florida, Serpa, Pollock, Nishikawa, Beshore, Ohata, Murashige, and Linda ($p<0.0001$). Murashige and Linda had non-significantly different zinc contents ($p=0.9445$) that were lower than Beshore, Nishikawa, Pollock, Serpa, Fuer Florida, and Peterson ($p<0.0001$). Serpa and Pollock had non-significantly different zinc content ($p=0.9445$), as did Nishikawa and Beshore ($p=0.9999$).

Copper was significantly larger in the BoothVIII cultivar (6.99mg/kg fresh wt.) than in any other cultivar ($p<0.0001$). Peterson (4.60mg/kg fresh wt.) had a significantly larger copper content compared to Serpa, Fuer Florida, Pollock, Beshore, Nishikawa, Murashige, Ohata and Linda ($p<0.0001$). Hawaiian avocado cultivars Murashige and Ohata did not have a significant difference in copper content ($p=0.0723$), however these two cultivars had a significantly lower copper content compared to Nishikawa, Beshore and Pollock ($p<0.0001$).

Table 2.1. Mineral profile (g/100g fresh wt.) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Beshore Mean	BoothVIII Mean	FuerFlorida Mean	Linda Mean	Murashige Mean	USDA Reference	P-value
Calcium	0.01±0.0^{C}	0.02±0.0^{B}	0.02±0.0^{A}	0.02±0.0^{A}	0.01±0.0^{CD}	0.012	<.0001
Magnesium	0.02±0.0^{C}	0.03±0.0^{C}	0.04±0.0^{A}	0.02±0.0^{C}	0.03±0.0^{C}	0.029	<.0001
Potassium	0.7±0.02^{C}	0.8±0.02^{A}	0.7±0.01^{C}	0.4±0.01^{G}	0.3±0.01^{H}	0.485	<.0001
Phosphorus	0.04±0.0^{DEF}	0.08±0.0^{A}	0.06±0.0^{B}	0.02±0.0^{G}	0.03±0.0^{FG}	0.052	<.0001

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Table 2.1. Mineral profile (g/100g fresh wt.) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Nishikawa Mean	Ohata Mean	Peterson Mean	Pollock Mean	Serpa Mean	USDA Reference	P-value
Calcium	0.01±0.0^{C}	0.01±0.0^{D}	0.02±0.0^{A}	0.01±0.0^{B}	0.01±0.0^{CD}	0.012	<.0001
Magnesium	0.03±0.0^{B}	0.02±0.01^{D}	0.04±0.0^{A}	0.03±0.0^{C}	0.03±0.0^{C}	0.029	<.0001
Potassium	0.60±0.0^{E}	0.40±0.01^{F}	0.7±0.0^{B}	0.40±0.0^{F}	0.6±0.0^{D}	0.485	<.0001
Phosphorus	0.04±0.0^{CDE}	0.03±0.00^{EF}	0.08±0.0^{A}	0.1±0.0^{BC}	0.1±0.0^{CD}	0.052	<.0001

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Table 2.2. Mineral profile (ppm, mg/kg fresh wt.) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Beshore Mean	BoothVIII Mean	FuerFlorida Mean	Linda Mean	Murashige Mean	USDA Reference	P-value
Manganese	2.0 ± 0.1^{B}	1.0 ± 0.1^{E}	1.0 ± 0.1^{DE}	1.3 ± 0.0^{C}	1.0 ± 0.1^{E}	1.42	<.0001
Zinc	4.0 ± 0.1^{E}	10.0 ± 0.1^{A}	6.2 ± 0.3^{C}	2.8 ± 0.0^{G}	3.0 ± 0.1^{FG}	6.4	<.0001
Sodium	17.0 ± 2.0^{CD}	5.0 ± 0.2^{F}	8.4 ± 1.1^{EF}	24.6 ± 1.0^{B}	34.0 ± 1.0	70.0	<.0001
Iron	7.0 ± 1.0^{B}	12.0 ± 0.8^{A}	12.0 ± 0.1^{A}	4.1 ± 0.1^{C}	4.0 ± 0.1^{C}	0.55	<.0001
Copper	3.0 ± 0.2^{DE}	7.0 ± 0.2^{A}	4.0 ± 0.1^{C}	1.3 ± 0.2^{F}	2.0 ± 0.2^{F}	1.9	<.0001

Different letters in the same row indicate statistically significant differences (Tukey's Test, $p<0.05$). Mean±SD, standard deviation (n=3).

Table 2.2. Mineral profile (ppm, mg/kg fresh wt.) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Nishikawa Mean	Ohata Mean	Peterson Mean	Pollock Mean	Serpa Mean	USDA Reference	P-value
Manganese	2.0 ± 0.0^{A}	1.0 ± 0.0^{EF}	1.0 ± 0.1^{CD}	1.0 ± 0.0^{F}	2.0 ± 0.0^{B}	1.42	<.0001
Zinc	4.0 ± 0.1^{E}	3.0 ± 0.2^{F}	8.0 ± 0.2^{B}	5.0 ± 0.1^{D}	5.3 ± 0.1^{D}	6.4	<.0001
Sodium	18.0 ± 2.0^{C}	36.0 ± 4.0^{A}	12.0 ± 2.0^{DE}	5.0 ± 0.8^{F}	21.0 ± 0.5^{BC}	70.0	<.0001
Iron	7.0 ± 0.6^{B}	5.0 ± 0.1^{C}	13.0 ± 0.4^{A}	8.0 ± 0.3^{B}	7.1 ± 0.1^{B}	0.55	<.0001
Copper	3.0 ± 0.1^{E}	2.0 ± 0.1^{F}	5.0 ± 0.2^{B}	3.0 ± 0.1^{D}	4.0 ± 0.1^{C}	1.9	<.0001

Different letters in the same row indicate statistically significant differences (Tukey's Test, $p<0.05$). Mean±SD, standard deviation (n=3).

3.3 *Lutein, Violaxanthin and Neoxanthin Profile*

The carotenoid profiles of avocado cultivars are presented in Table 3. Lutein content was significantly higher in the Cameroon grown Fuer Florida cultivar (0.538mg/g fresh wt.) compared to the other avocado cultivars ($p<0.0001$). Lutein content was not significantly different between Peterson and Serpa ($p=0.0710$) as well as between Nishikawa and Linda ($p=1.0$), Nishikawa and Ohata ($p=1.0$), and Nishikawa and BoothVIII ($p=0.5551$). Murashige had a lutein content of 0.03mg/g fresh wt. which was significantly lower than Pollock ($p=0.0112$) and Peterson ($p=0.0002$).

The carotenoid violaxanthin was not present at detectable levels in the Hawaii grown cultivars Beshore and Nishikawa. The violaxathin content was larger in Fuer Florida (0.075mg/g fresh wt.) c=ompared to Murashige (0.015mg/g fresh wt.) ($p=0.02$). Nishikawa, Beshore, Murashige, Linda and Pollock has non-significantly different violaxanthin content ($p>0.06$). Serpa (0.067mg/g fresh wt.), BoothVIII (0.061mg/g fresh wt.) and Peterson (0.036mg/g fresh wt.) also had violaxanthin content that was not significantly different ($p>0.5$).

The carotenoid neoxanthin was not present at detectable levels in the Hawaii grown Beshore cultivar. Neoxanthin content in Fuer Florida was 0.227mg/g fresh wt. This was significantly larger than the neoxanthin content in Serpa ($p=0.0139$), Pollock ($p=0.0179$), Peterson ($p=0.0318$) and Linda ($p=0.0374$). The neoxanthin content did not significantly differ between Fuer Florida and BoothVIII, Ohata, Murashige, and Nishikawa ($p>0.06$). Neoxanthin content also did not significantly differ among Beshore, Serpa, Pollock, Peterson and Linda ($p=1.0$)

Table 3.1. Lutein, violaxanthin, and neoxanthin (mg/g sample wet basis) profile of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Beshore Mean	BoothVIII Mean	FuerFlorida Mean	Linda Mean	Murashige Mean	USDA Reference	P-value
Lutein	0.05±0.0^{C}	0.15±0.01^{BC}	0.54±0.06^{A}	0.06±0.0^{C}	0.03±0.03^{C}	0.00271	<.0001
Violaxanthin	0.0±0.0^{C}	0.06±0.04^{AB}	0.08±0.03^{A}	0.0±0.0^{ABC}	0.03±0.03^{BC}	-	0.0003
Neoxanthin	0.0±0.0^{B}	0.08±0.03^{AB}	0.23±0.08^{A}	0.07±0.1^{B}	0.1±0.09^{AB}	-	0.0071

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Table 3.1. Lutein, violaxanthin, and neoxanthin profile (mg/g sample wet basis) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Nishikawa Mean	Ohata Mean	Peterson Mean	Pollock Mean	Serpa Mean	USDA Reference	P-value
Lutein	0.06±0.01^{C}	0.05±0.1^{C}	0.3±0.01^{B}	0.2±0.03^{B}	0.2±0.1^{BC}	0.00271	<.0001
Violaxanthin	0.0±0.0^{C}	0.03±0.03^{ABC}	0.04±0.01^{ABC}	0.03±0.003^{ABC}	0.1±0.0^{AB}	-	0.0003
Neoxanthin	0.1±0.05^{AB}	0.08±0.06^{AB}	0.1±0.01^{B}	0.05±0.005^{B}	0.1±0.0^{B}	-	0.0071

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

3.4 *Fatty Acid Profile*

The theoretical yield of oil from the avocado tissue was found to be 100mg/g of sample. This was observed for cultivars Beshore, BoothVIII, Linda, Murashige, Ohata, Peterson, Pollock and Serpa. Cultivars Nishikawa and Fuer Florida had a theoretical yield of oil of 200mg/g sample. Seven fatty acids were quantified in this analysis; they were linolenic acid, linoleic acid, oleic acid, palmitoleic acid, stearic acid, palmitic acid and myristic acid. These fatty acids were summed together to calculate the total amount of fatty acids which was then compared to the theoretical oil yields. Beshore had a fatty acid sum of 73mg, BoothVIII had a sum of 66mg and Linda had 63mg. Murashige had a sum of 68mg, Ohata had 76mg and Peterson had 66mg. Pollock had 52mg and Serpa had a sum of 64mg. The sum of the individual fatty acids in Nishikawa was 166mg and for Fuer Florida it was 130mg.

The saturated fatty acid profiles of avocado cultivars are presented in Table 4. The amount of myristic acid (C_{14}) was not significantly different among all ten avocado varieties ($p>0.1$). Myristic acid was largest in the Hawaii cultivar Nishikawa (0.33mg/g fresh wt.) and the Cameroon cultivar BoothVIII (0.33mg/g fresh wt.). However, this content was not significantly different from the smallest content which was in the Pollock cultivar (0.15mg/g fresh wt.).

The palmitic acid (C_{16}) content was significantly larger in the Nishikawa cultivar (35.72mg/g fresh wt.) compared to cultivars Pollock ($p=0.0024$), Ohata ($p=0.0087$), Peterson ($p=0.0147$), Linda ($p=0.0161$), and Murashige ($p=0.0224$). Nishikawa did not significantly differ in its palmitic acid content compared to Serpa, Beshore, BoothVIII, and Fuer Florida ($p>0.1$). Palmitic acid also did not significantly differ between Pollock (10.46 mg/g fresh wt.), Ohata (13.48 mg/g fresh wt.), Peterson (14.71 mg/g fresh wt.), Linda (14.93 mg/g fresh wt.) and Murashige (15.71 mg/g fresh wt.) ($p>0.1$).

The Cameroon cultivar Fuer Florida had a stearic acid (C_{18}) content of 2.68mg/g fresh wt. Fuer Florida had a larger content of this saturated fatty acid compared to Pollock ($p=0.011$), Ohata ($p=0.014$) and Murashige ($p=0.029$). Nishikawa had a stearic acid content of 2.67mg/g fresh wt., which was larger than Pollock ($p=0.013$), Ohata ($p=0.015$) and Murashige ($p=0.03$). Cultivars Murashige (1.02mg/g fresh wt.), Ohata (0.87mg/g fresh wt.) and Pollock (0.84mg/g fresh wt.) had stearic acid contents that were not significantly different from each other ($p>0.5$).

The monounsaturated fatty acid profiles of the ten avocado cultivars are presented in Table 4.1. Palmitoleic acid ($C_{16:1}$) content was not significantly different among all the ten avocado cultivars ($p>0.06$). The Nishikawa cultivar had the largest content at 21.90mg/g fresh wt. This however was not different from the Ohata cultivar which had 9.29mg/g fresh wt. ($p>0.06$). Cultivars Nishikawa and Ohata were both from South Kona, Hawaii. The PLA content did not differ among cultivars from Hawaii and Cameroon, nor did they differ among each other ($p>0.06$).

Oleic acid ($C_{18:1}$) was significantly higher in Nishikawa (39.82mg/g fresh wt.) compared to Pollock ($p=0.0028$), Peterson ($p=0.0392$), and BoothVIII ($p=0.0475$). The Cameroon cultivar Fuer Florida (34.68mg/g fresh wt.) had a higher content of oleic acid compared to the Cameroon cultivar Pollock (10.82mg/g fresh wt.) ($p=0.018$). Oleic acid content did not significantly differ among the following cultivars: Nishikawa, Fuer Florida, Serpa, Beshore, Linda, Ohata, and Murashige ($p>0.09$).

The polyunsaturated fatty acid profiles of the ten avocado cultivars are presented in Table 4.2. Linoleic acid ($C_{18:2}$) content was significantly higher in the Nishikawa cultivar (44.8mg/g fresh wt.) compared to the cultivars Pollock ($p=0.001$), Linda ($p=0.0023$), Peterson ($p=0.0185$) and Fuer Florida ($p=0.0417$). Nishikawa did not differ in its linoleic acid content compared to cultivars Serpa (20.5mg/g fresh wt.), Ohata (19.81mg/g fresh wt.), Beshore (29.95mg/g fresh wt.), Murashige (19.25mg/g fresh wt.) and BoothVIII (18.76mg/g fresh wt.) ($p>0.06$).

Linolenic acid ($C_{18:3}$) content was significantly higher in the Nishikawa cultivar (2.23mg/g fresh wt.) compared to Linda ($p=0.0006$), Pollock ($p=0.0012$), and Fuer Florida ($p=0.0083$). The Hawaiian Serpa (1.77mg/g fresh wt.) had a larger linolenic acid content compared to Linda ($p=0.0125$) and Pollock ($p=0.0253$). The LA content in Linda (0.39mg/g fresh wt.) and Pollock (0.50mg/g fresh wt.) was not significantly different from each other ($p=1.0$). Cultivars Ohata (1.41mg/g fresh wt.), Beshore (1.37mg/g fresh wt.), Murashige (1.22mg/g fresh wt.), BoothVIII (1.18mg/g fresh wt.) and Peterson (1.15mg/g fresh wt.) did not significantly differ in their linolenic acid content ($p>0.1$

Table 4.1. Saturated fatty acid profile (mg/g sample wet basis) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Beshore Mean	BoothVIII Mean	FuerFlorida Mean	Linda Mean	Murashige Mean	USDA Reference	P-value
Myristic acid	0.3±0.1	0.3±0.0	0.3±0.0	0.2±0.0	0.2±0.01	0.00	0.0994
Palmitic acid	24.0±5.0^{AB}	24.0±3.0^{AB}	24.0±2.0^{AB}	15.0±1.0^{B}	16.0±1.0^{B}	20.75	0.0033
Stearic acid	2.0±0.6^{AB}	2.1±0.2^{AB}	3.0±0.2^{A}	1.0±0.1^{B}	1.0±0.0^{B}	0.49	0.0013

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Table 4.1. Saturated fatty acid profile (mg/g sample wet basis) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Nishikawa Mean	Ohata Mean	Peterson Mean	Pollock Mean	Serpa Mean	USDA Reference	P-value
Myristic acid	0.3±0.2	0.2±0.0	0.2±0.1	0.2±0.0	0.2±0.1	0.00	0.0994
Palmitic acid	36.0±17.0^{A}	14.0±2.0^{B}	15.0±4.0^{B}	11.0±1.0^{B}	20.0±7.0^{AB}	20.75	0.0033
Stearic acid	3.0±1.0^{A}	1.0±0.1^{B}	2.0±1.0^{AB}	1.0±0.2^{B}	2.0±1.0^{AB}	0.49	0.0013

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Table 4.2. Monounsaturated fatty acid profile (mg/g sample wet basis) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Beshore Mean	BoothVIII Mean	FuerFlorida Mean	Linda Mean	Murashige Mean	USDA Reference	P-value
Oleic acid	24.0±6.0^{ABC}	19.0±2.0^{BC}	35.0±4.0^{AB}	24.0±2.0^{ABC}	21.0±1.0^{ABC}	90.66	0.0048
Palmitoleic acid	13.0±3.0	22.0±1.0	18.0±2.0	17.0±2.0	10.0±0.4	6.98	0.0091

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Table 4.2. Monounsaturated fatty acid profile (mg/g sample wet basis) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Nishikawa	Ohata	Peterson	Pollock	Serpa	USDA	P-value
	Mean	Mean	Mean	Mean	Mean	Reference	
Oleic acid	40.0±19.0^{A}	21.0±3.0^{ABC}	18.0±5.0^{BC}	11.0±1.0^{C}	27.0±9.0^{ABC}	90.66	0.0048
Palmitoleic acid	22.0±12.0	9.0±1.0	11.0±4.0	9.0±2.0	12.0±5.0	6.98	0.0091

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Table 4.3. Polyunsaturated fatty acid profile (mg/g sample wet basis) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Beshore	BoothVIII	FuerFlorida	Linda	Murashige	USDA	P-value
	Mean	Mean	Mean	Mean	Mean	Reference	
Linoleic acid	30.0±8.0^{AB}	19.0±0.6^{AB}	17.0±1.0^{B}	7.0±0.7^{B}	19.0±1.0^{AB}	16.74	0.0025
α -linolenic acid	1.0±0.3^{ABC}	1.0±0.01^{ABC}	1.0±0.0^{BC}	0.4±0.1^{C}	1.0±0.1^{ABC}	1.11	0.0006

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Table 4.3. Polyunsaturated fatty acid profile (mg/g sample wet basis) of Hawaii and Cameroon grown avocados, all values are on a fresh wt. basis.

	Cultivar						
	Nishikawa	Ohata	Peterson	Pollock	Serpa	USDA	P-value
	Mean	Mean	Mean	Mean	Mean	Reference	
Linoleic acid	45.0±27.0^{A}	20.0±4.0^{AB}	14.0±5.0^{B}	5.0±1.0^{B}	21.0±7.0^{AB}	16.74	0.0025
α -linolenic acid	2.0±1.0^{A}	1.4±0.0^{ABC}	1.2±0.4^{ABC}	1.0±0.2^{C}	2.0±0.6^{AB}	1.11	0.0006

Different letters in the same row indicate statistically significant differences (Tukey's Test, p<0.05). Mean±SD, standard deviation (n=3).

Chapter 4
Discussion and Conclusions

4.1 Discussion

Avocados are a well-adapted fruit tree with potentially high crop yields. The fruit has spread beyond its native Latin American and Caribbean regions and is widely cultivated in tropical climates in Asia, Africa, North American and Europe. Avocados are liked among consumers for their butter and nut-like flavor. Aside from being tasty, avocados are a nutrient dense food. The mixed composition of the fruit includes many essential nutrients (i.e. fiber, lipid, unsaturated fatty acids, antioxidants, minerals) which make avocados a healthy dietary choice. For example, lipids provide the body with energy and essential fatty acids (i.e. omega-3 and omega-6 fatty acids) needed for optimal function. Lipids also aid in the absorption of fat-soluble vitamins and carotenoids in the gastrointestinal tract. However, the nutrient profile of avocados is complicated by the variation in nutrient content due to growing region (i.e. elevation, temperature, rainfall), cultivation practices (i.e. fertilization, orchard maintance), time of harvest, cultivars and postharvest handling and supply (Bill *et* al., 2014; Lu *et al*., 2009; Peraza-Magallanes *et al*., 2017; Pedreschi *et al*., 2016; Wang *et al*., 2010).

The data from this research confirms avocados as being a rich source of lipids and thereby an energy dense food. The data also revealed variation in the total lipid content and individual fatty acid content among the ten unique cultivars. According to the USDA nutrient database (2016), the average commercial avocado has 14.66g of total lipid per 100g fresh wt. which was considerably less than majority of the avocados examined in this study. Nishikawa and Fuer Florida had nearly double the USDA value at 35.3 and 30.0 g/100 g fresh wt., respectively. Of the ten varieties examined, Peterson, Ohata and Pollock had total lipid content similar to the USDA reported value at 18.4, 17.3, and 14.4 g/100g fresh wt., respectively. Lipid content varied among cultivars more so than growing region and harvest, making it seem the genetic background had a stronger influence than environment. Nishikawa and Ohata were both grown in South Kona, Hawaii under the same growing conditions (i.e. elevation and temperature) yet significantly differed in fat content. Nishikawa had a statistically similar fat content to Fuer Florida which was grown in Cameroon, Central Africa. Peterson and Pollock cultivars were also grown in Cameroon under the same conditions as Fuer Florida yet differed from Fuer Florida in lipid content. Peterson and Pollock were similar to Hawaii grown Ohata in their lipid content.

Lipids have an important physiological role in the human body. Lipids can exhibit hormone-like functions such as altering blood pressure and platelet aggregation as well as heighten immunological responses (Gropper and Smith, 2013). Specific structural functions of lipids include composing cell and organelle membranes (e.g. phospholipid bilayer, glycolipids) as well as serving as precursors to corticosteroid hormones and coenzyme Q, a mediator of the electron transport chain leading to ATP synthesis (Gropper and Smith, 2013).

The fatty acid composition of the avocados consists of both saturated and unsaturated fatty acids. Oleic acid is an MUFA associated with healthy outcomes such as normal blood pressure and lipid profile (Teres *et al.*, 2008). Oleic acid was found in all ten avocado varieties. Nishikawa had nearly 40mg/g fresh wt. and Fuer Florida had 35mg/g fresh wt. Pollock had the least OA content with 10.8mg/g fresh wt. Oleic acid content among the Hawaii and Cameroon cultivars was lower than values reported in the literature (Meyer *et al*., 2008; USDA, 2016). This could be attributed to different avocado varieties being tested. Peraza-Magallanes *et al*. (2016) found variability among five cultivars that grow in the same region in Northwest Mexico. The USDA nutrient data (2016) is based on a non-specific variety and Meyer *et al*. (2008) examined the Haas cultivar grown in Malaga, Spain.

Linoleic acid is an omega-6 fatty acid that is essential in the diet. Humans do not biologically synthesize this PUFA and so it needs to be consumed. This study found linoleic acid present in all ten cultivars. Nishikawa was among the highest with 44.8mg/g fresh wt., which was significantly more compared to Linda. Both fruits were grown in the same region under similar conditions. Linda had similar linoleic acid content as three of the African avocados. Linoleic acid content in seven of the examined cultivars was higher compared to the reported linoleic acid content in the USDA database (2016), 16.7mg/g fresh wt. of commercially available raw avocados. Linoleic acid is needed by the body to make y-linolenic acid, eicosatrienoic acid, arachidonic acid and docosapentaenoic acid (Gropper and Smith, 2013). Linoleic acid is also needed to make potent bioactive compounds such as prostaglandins, thromboxanes and leukotrienes. These compounds may work in concert to lower serum cholesterol and prevent and control degenerative cardiovascular disease (Gropper and Smith, 2013).

The variability in the quantity of individual fatty acids was also seen in the study by Pedreschi *et al*. (2016). In their work, the Hass cultivar from four different growers in Chile was found to have different fatty acid profiles. The researchers attributed the discrepancies to the stage of maturity at harvest, post – harvest handling techniques, and agricultural conditions. Although the difference was non-significant, Pedreschi *et al*. (2016) assert the growing environment and handling of the fruit as influential factors in the fatty acid composition. Previous research by Plaza *et al*. (2009) supports the influence storage has on fatty acid composition. The fatty acids were found to decrease due to oxidative degradation in samples stored at 8°C for 8 days, as well as those stored in air, nitrogen and in vacuum packaging. The vacuum – packed avocados had the least amount of lipid oxidation (Plaza *et al*., 2009).

Variability in the total lipid content and fatty acid profile was also seen among avocados grown in Japan and those imported into the country (Takenaga *et al.*, 2008). Although the variability was not significant, the locally grown Bacon cultivar had more lipid than the imported Hass cultivar. This was also seen in the oleic acid content, with the locally grown Bacon and Fuerte cultivars having more than the imported Hass. The researchers attributed the difference to fruit variety and damage incurred from importation (Takenaga *et al.*, 2008). Variability is also attributed to the specific time of year the fruit is picked and the ripeness of the avocado when its consumed (Ozdemir and Topuz, 2004). The total lipid content and fatty acid profile of two cultivars changed as the fruits were picked later in the season. Lipid content and oleic acid content increased in fruits harvested in January compared to those harvested in November of the previous year. Palmitic acid and α-linolenic acid decreased longer into the fruit season (Ozdemir and Topuz, 2004). The researchers concluded avocados allowed to mature longer on the tree will yield a higher lipid content and altered profile, increasing the nutritional benefit of the fruit.

Carotenoids are a diverse group of phytochemicals, some of which can be made into the fat-soluble vitamin A. Lutein is a special type of carotenoid called an oxygenated carotenoid that does not form Vit. A. Lutein is crucial in maintaining healthy eyesight, being used in synthesizing rhodopsin and other light receptor pigments. Lutein has also been found to be protective against cataract and age-related macular degeneration (Gropper and Smith, 2013). The quantification of carotenoids in the edible portion of avocados is of interest not only because of potential health benefits but also to understand the

bright yellow-green pigmentation of the fruit. Lutein and neoxanthin typically present themselves in the natural yellow colors of foods, while violaxanthin physically appears orange.

The physical appearance of the flesh of the cultivars were shades of yellow and green. Among the Hawaiian cultivars, Nishikawa and Beshore physically appeared more yellow. Beshore had a darker mustard yellow compared to Nishikawa's brighter yellow. Serpa was also bright yellow although, compared to Nishikawa was whiter and lighter. Murashige, Ohata and Linda appeared to have less intense yellow coloring. Cameroon Fuer Florida had the most pronounced green color compared to all the avocado cultivars. Fuer Florida also appeared brighter yellow compared to the other Cameroon cultivars.

Lutein was found in all ten cultivars, with Fuer Florida having the most at 0.54mg/g fresh wt. The lowest lutein content was in Murashige at 0.03mg/g fresh wt., although this was not significantly lower than most of the fruits tested. The lutein values found in this study were higher than those reported for the Hass cultivar. Lu *et al.* (2005) found the lutein content in Hass avocados to be 0.003mg/g fresh wt. Ashton *et al*. (2006) found lutein content to be 0.002mg/g fresh wt. in Hass avocados. In the USDA database, lutein content is measured alongside zeaxanthin and was reported as 0.003mg/g fresh wt. (2016).

Fuer Florida was also high in violaxanthin and neoxanthin. This was seen physically, with Fuer Florida appearing brighter and with more dominate yellow and green colors compared to the other cultivars. Beshore was found to have no violaxanthin and neoxanthiin in detectable amounts. This was contradictory since the flesh had a pronounced yellow color. Linda had low violaxanthin and neoxanthin content. This was supported by the color of the flesh which was lighter and dull compared to Fuer Florida. Overall, the coloration varied among the avocado cultivars however, the variation was not completely reflective of the carotenoid content. This was evidence by Beshore and Nishikawa, both of which appeared to have higher carotenoid content than what was found. Color and carotenoid content were reflective of each other in regards to Fuer Florida, with this cultivar having high lutein, neoxanthin and violaxanthin content as well as having pronounced green and yellow pigmentation.

A significant consideration regarding the carotenoid content is sample processing which involves cutting the fruit and freeze drying it. Plaza *et al*. (2009) reported that the disruption of cell membranes releases the antioxidants in the peel and mesocarp. Freeze drying also causes the carotenoids to become oxidized. These procedures result in experimental values misrepresenting the actual value that would be consumed and present for biological use. Lyophilization was important in this study to make the avocado samples "shelf-stable" and preserved, as well as to calculate the moisture content with as little damage to the integrity of the fruit.

Carotenoid content was also found to vary within an avocado according to tissue position (Ashton *et al.*, 2006). They found more pigmentation in the outer tissue near the skin compared to inner tissue near the seed. The mesocarp with a greener pigment had greater carotenoids and chlorophyll. Carotenoid content also declined in the flesh over time, with more in the unripe fruits. This was a unique feature as other studies found carotenoid to be significantly correlated to oil content with oil content increasing with maturity (Jacobo-Velazquez *et* al., 2012; Lu *et al*., 2009).

Identifying the mineral profile was important to understanding the variability among varieties and place of growth. Individual mineral content is essential in relating foods to expected health outcomes associated with their consumption. Mineral content is influenced not only by fruit genetics but equally by agronomic management. Plant nutrition practices (e.g. fertilizer, availability of sodium, calcium and nitrogen) is a crucial pre-harvest factor with profound effects ranging from nutrient content to susceptibility to physiological disorders. Mineral content, and nutrient content in general are also significantly influenced by climate (temp. and rainfall) and soil along with pruning and rootsock practices (Bill et al., 2014).

In this study, Cameroon cultivars were observed to have a higher total mineral content compared to Hawaii cultivars. However, this result could not be used to predict the outcomes for the individual mineral content. We observed no fixed behavior among the examined cultivars. None of them consistently presented the highest values for the minerals profiled. Potassium is reported to help prevent hypertension by normalizing blood pressure (McDonough *et al*., 2017; Shenoy *et al*., 2010). The AI for potassium for adult (≥19yrs) males and females is 4.7g/day (Gropper and Smith, 2013). The ten avocado

cultivars profiled in this study had potassium upwards of 0.3g/100g fresh wt.; the exact amount varied among the cultivars. African avocados differed from each other and from the Hawaiian avocados. The Hawaiian avocados similarly, differing from each other and from the Cameroon avocados. What was observed was majority of the Cameroon grown varieties had significantly higher potassium content compared to the Hawaiian varieties.

Cameroonian BoothVIII (0.82g/100g fresh wt.) and Peterson (0.73g/100g fresh wt.) had the most potassium, while Hawaiian Linda (0.36g/100g fresh wt.) and Murashige (0.31g/100g fresh wt.) had the least. These four varieties were significantly different from each other. Fuer Florida (0.7g/100g fresh wt.) and Beshore (0.68g/100g fresh wt.) had similar quantities, with the former being from Africa and the latter being from Hawaii. African Pollock (0.41g/100g fresh wt.) and Hawaiian Ohata (0.41g/100g fresh wt.) also had similar quantities. The value of potassium found in this study was in general agreement to that in the USDA database. Potassium content in a non-specific commercial avocado cultivar was 0.485g/100g fresh wt. (USDA, 2016).

Variability among the cultivars was also seen in the sodium content. As a collective whole, the Hawaiian varieties had significantly more sodium compared to the Cameroon avocados. Ohata and Murashige had the highest content with 35.8 and 33.8mg/kg fresh wt. respectively. All four African varieties had the lowest content; Peterson had 11.9mg/kg fresh wt., Fuer Florida had 8.3mg/kg fresh wt., BoothVIII had 5.4mg/kg fresh wt. and Pollock had 4.7mg/kg fresh wt. There was variability among the cultivars from the same region. Sodium content in the USDA database (2016) was 70mg/kg fresh wt. The sodium content found in this study was comparatively lower than the USDA reported value.

Mineral content also varied among four avocado cultivars (Arona, Fuerte, Hass and Orotava) grown in the Canary Islands (Hardisson *et al*., 2001). The Hass variety was significantly higher in sodium, potassium, calcium, magnesium and phosphorus, while Fuerte was higher in iron, copper, zinc, manganese and boron. Furthermore, the Hass variety differed in mineral content depending where on the island it was grown. Hass from the northern region was higher in potassium, calcium and phosphorus, while Hass from the southern region was higher in sodium and magnesium. This is significant as it shows nutrient content is dependent on external factors rather than genetic variations.

The researchers proposed the variances were due to the nature of the soils, climate, crop system and harvesting methods. The area of origin had a significant effect (Hardisson *et al*., 2001).

Post-harvest management practices are crucial in maintaining the quality of harvested fruit. Once an avocado has been picked, the nutrient content of the fruit can no longer be improved. This makes the route from the farm to the consumer's table crucial. Avocados are one of the fastest ripening fruits when picked at a mature state, needing on average a week for the skin and flesh to become penetrable. This feature makes it easy for the fruit to be damaged and susceptible to decay from microbial contamination (Bill *et al*., 2014). Appropriate storage temperatures (2-7°C) were found to delay the ripening of avocados as well as inhibit bacterial growth. However, post-harvest practices are at the discretion of the distributor with some opting out due to cost. To overcome these problems, fruits are picked earlier when they are firmer (Bill *et al*., 2014).

In this study, avocados were picked at mature stages in their development. The fruits were handled by the farmer who harvested them and by the researcher who collected the data. There was no cold temp. storage nor long duration in warm to hot temperatures. Fruits were gathered in such a manner to resemble "farm fresh", going from the tree to the consumer's table with little handling in between. Avocados were ripened at room temp similar to how consumers would ripen their fruits. Aside from growing regions and techniques, typical variabilities seen between different farms and cultivation styles, the major distinction between the avocados was variety. The data collected suggests the innate genetic variability, which distinguishes the cultivars, greatly influences the nutrient composition of the fruit. The nutrient profiles of the ten avocado cultivars provided here will expand the nutritive information available for the fruit. Furthermore, comparing Hawaii-grown and Cameroon-grown cultivars helps in characterizing which variety(s) is/are more appropriate in regards to nutrition and commercial production. Overall, the Hawaii grown Nishikawa cultivar is desirable due to its high total lipid content and fatty acid profile. The cultivar is also smaller in size and therefore more manageable to transport and store.

A significant limitation of this study was the unconventional method moisture content was done and calculated. Sample drying was not done sequentially. The Hawaiian avocado samples were freeze dried in early May 2016 in Hawaii, then flown to Yaounde, Cameroon later that month. Cameroon

cultivars were harvested in early June 2016 and freeze dried within a week. Air drying was done in the second week of June to all freeze dried cultivars to remove the residual 10% moisture content.

In this disrupted drying method, freeze dried samples were air dried. The loss of water was the summation of water loss during freeze drying and water loss during air drying. There is a possibility the reported moisture content is not accurate. The moisture content may be lower than the actual moisture content because not all the water was removed. A lower moisture content would present a nutrient quantity higher on a wet weight basis. If this is the situation then the nutrient contents reported in this research are more than what is present in the cultivar.

The reason drying and subsequently moisture content determination was done this way was because we wanted to analyze the Hawaiian and Cameroonian cultivars simultaneously. We wanted the samples to undergo the same experiments so the results would be comparable. This is why the Hawaiian cultivars had their proximate nutrient analyses done in Cameroon, and the Cameroonian cultivars had their bioactive compound analyses done in Hawaii. The moisture content found in this study ranged from 80%% moisture in the Ohata to 59.0% moisture in Nishikawa. Fuer Florida had 64% moisture while BoothVIII had 67% and Peterson had 64%. The moisture content reported in the USDA database is 73%. The interrupted method of moisture profiling resulted in values similar to that in the USDA database. Linda, Murashige and Peterson and Serpa had percent moisture in the high 60 to low 70s.

4.2 Conclusion

Several factors influence the nutrient content of foods such as genetic background, agronomic techniques including fertilization, cultivation, time of harvest, and post-harvest practices. The data collected in this study revealed similarities and differences in the nutrient profiles of avocado cultivars grown in Hawaii and Cameroon. The overall findings strongly suggest not all cultivars are the same. The variability observed among the ten unique cultivars will help consumers understand the complexity of food composition and the importance of knowing the food being consumed. Currently, the USDA database is the reference most professionals refer to when looking up nutrient values for specific foods. Although this

resource is exhaustive in many regards, the data for raw avocados is misleading due to the non-specific varieties being referenced. The data collected in this study will help to expand the nutritional information on avocados and give a more complete understanding of the potential health benefits of this fruit. Furthermore, this study is unique in its international comparison of the same fruit. Previously, there was no research examining the nutrient differences between Hawaii grown and Cameroon grown avocados.

From a nutritive and commercial perspective, the Hawaii grown Nishikawa cultivar is an avocado worthy of devoting precious resources. Nishikawa had significantly more lipid content in its mesocarp which is a feature consumers and chefs in Hawaii prefer (Barber et al., 2008). In regards to unsaturated fats, Nishikawa was among the highest for PUFAs omega-3 linolenic acid and omega-6 linoleic acid. These fatty acids are essential fats since the human body cannot synthesize them and so are required in the human diet. Committing to the growth and promotion of the appropriate avocado cultivars ensures the availability of fruits most advantageous for overall health. The founder of modern medicine, Hippocrates believed nutrition was a form of medical treatment with the latter being incomplete without the incorporation of eating foods with potential health benefits. Food is a significant influence on the physiological functioning of the body and its ability to perform optimally and maintain crucial homeostasis. Neglecting nutrition initiates a cascade of dangerous repercussions that may have been preventable. This underlies the need to provide and encourage consumption of food, such as avocados, which possess chemical compounds potentially beneficial to human health.

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