UNIVERSIDADE DE BRASÍLIA
FACULDADE DE AGRONOMIA E MEDICINA VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

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TESE DE DOUTORADO SUBMETIDA AO PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA, COMO PARTE DOS REQUISITOS NECESSÁRIOS À OBTENÇÃO DO GRAU DE DOUTOR EM AGRONOMIA.

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RESUMO

Avaliaram-se os efeitos de diferentes cultivares e estágio de maturação do abacate, fruto brasileiro, (Persea Americana, Mill) no teor de óleo, matéria seca e composição de ácidos graxos. Cinco cultivares de abacate, fruto, incluindo Hass, Quintal, Fortuna, Breda e Margarida a, foram colhidos em três e quatro diferentes estágios de maturação, de junho a setembro de 2015, de acordo com o padrão de colheita da Fazenda Tsuge, em Rio Paranaíba, Minas Gerais, Brasil. O teor de matéria seca e O teor de óleo aumentou significativamente em todos os estágios de maturação, todas as cultivares.Em todos os estágios de maturação, todas as cultivares, ácido oléico, palmítico, linoléico e palmitoleico foram os principais ácidos graxos. A particularidade do presente trabalho no Brasil aparece na nova metodologia de extração utilizada, sem uso de (enzima, CO_2 supercrítico, hidrólise ácida, alcalina, solvente, aquecimento ou adição de água); Ele depende da separação do óleo usando centrífuga e misturando alternativamente. O óleo extraído é de alta qualidade; uma cor clara amarela ou verde, agradável e atraente, é o Óleo de Abacate Extra Virgem (EVAO), pois não precisa ser refinado.

Palavras-chave:Hass, Breda, Margarida, Quintal, Fortuna.

ABSTRACT

The effects of different cultivars and ripening stages of Brazilian avocado fruits (Persea Americana, Mill) on oil content, dry matter, and fatty acid composition were evaluated. Five cultivars of avocado fruits including Hass, Quintal, Fortuna, Breda and Margarida were harvested at three to four different ripening stages from June to September, 2015, according to Tsuge Farm picking standard in Rio Paranaíba city, Minas Gerais State, Brazil. Dry matter and oil content increased significantly during ripening stages in all cultivars. In all ripening stages, of all cultivars, oleic, palmitic, linoleic and palmitoleic acids were the major fatty acids. The particularity of the present work in Brazil appears in the new extraction methodology used, without usage of (enzyme, supercritical CO2, acid hydrolysis, alkaline, solvent, heating, or add water); it relies on the separation of oil using centrifugal and mixing alternatively. The extracted oil is of high quality; a nice and attractive clear yellow or green color is Extra-Virgin Avocado Oil (EVAO) as it does not need to be refined.

Key Word: Hass, Breda, Margarida, Quintal, Fortuna.

1. GENERAL INTRODUCTION

Avocado (*Persea americana Mill*) is an oil-rich, highly nutritious fruit which is abundant in Brazil but has a very small local market. The avocado oil contains all the beneficial attributes of the fruit, which makes it a very valuable product. It contains high amounts of the anti-cholesterol agent beta-sitosterol, a wide variety of vitamins and antioxidants, and other plant chemicals, which impart beneficial functional properties on humans.

The pulp contains up to 30% of the oil (based on fresh weight). It is rich in monounsaturated fatty acids, and has nutritional properties similar to those of olive oil. However, there is no widespread commercial method for oil recovery from avocado pulp.

The process for recovering oil from ripe avocados is a mechanical extraction, similar to that used for olive oil extraction, with plus the steps of removing the skin and stone (seed). The flesh is then ground to a paste and malaxed for 40-60 minutes at 45-50°C. This is a higher malaxing temperature than the one used for olive oil extraction, but it is still considered to be cold-pressed extraction for avocado oil. The slightly higher temperature aids the extraction of the oil from the oil-containing cells and does not affect the quality of the oil. The oil and water phases are separated from the pulp using a high-speed decanting centrifuge, and then the oil is separated from the water in final polishing centrifuges, or by heating the pulp and pressing out the oil, solvents, enzymes, supercritical CO_2. The cold press and supercritical CO_2 methods are the two best options to consider for processing avocado oil. The pulp from the decanting centrifuge and waste skin/seeds are returned to orchards for soil conditioning and mulch; it can also be used as animal feed. Seeds may be used for biofuel extraction.

Avocado oil for cosmetics is traditionally extracted with solvents at elevated temperatures. After extraction, the oil for application in skin care products is usually refined, bleached, and deodorized, resulting in odorless yellow oil.

The oil can be produced as a virgin oil or as a refined oil suited for various food preparation. The unsaponifiable fraction of the oil represents another major profitable market as used in the cosmetic and pharmaceutical industries, which has been the traditional end-user of avocado oil.

The use of immature fruits to produce oil with higher unsaponifiable content and phytochemicals is an interesting area to pursue for capsule production.

Important Concepts Regarding Avocado Oil:

1- Quantitative Factors:
   The more the quantity of the original oil in the Avocado fruits would result in the higher rate of oil extracted. Using efficient method such as the decanter and the centrifuge may enhance the quantity of oil extracted; especially when parameter of extraction method is optimized.
2- Qualitative Factors:
   Avocado oil quality can be improved by implementing low temperature extraction method, less than 27°C with no oxygen availability during mixing of the Avocado pulp and oil extraction process by pumping the N_2 gas inside the system. Hence the resulting oil would be low in [FFA] and Peroxide values.
3- The resulting oil will be healthy and natural, presenting higher marketing values. Hence may contribute in the economical progress of the Avocado oil producing country such as Brazil.

2. LITERATURE REVIEW

2.1. Avocado in the World

The avocado tree is a fruit plant originated in the Americas, especially Mexico and Central America, belonging to the *Lauraceae* family and *Persea* genus (Maranca, 1980; Koller, 1992).

It is one of the most productive plants per unit of cultivated area (15-40 ton/hectare) in Brazil. Many varieties of avocado are found in different regions of Brazil, whose fruits have varied chemical composition, especially in terms of levels of lipids in the pulp. Fruits with high levels of lipids in the pulp can be important raw material for oil extraction (Tango; Turatti, 1992).

Mexico is the country that leads the production of avocados in the world; Brazil is one of the largest producers in the world as shown in table 1. The state of São Paulo is the largest domestic producer, accounting for more than 50% of production. The state of Minas Gerais is the second, followed by Paraná and Espírito Santo as shown in Figure 1. (AGRIANUAL, 2016).

Avocado fruits are used for oil extraction when they are mature or have showing soft consistency. In this stage they have higher levels of oil, facilitate the separation of the peel and core, and still facilitate processing to obtain the oil (Tango and Turatti, 1992).

Lucchesi (1975) reported that the oil content in avocado pulp can be less than 2% during the first two months of fruit standing on the tree, then increases slowly to the final stage, than fast reaching up to 35% of the pulp.

Oil levels in “Hass” avocado flesh can increase 1–2% early in a season to >30% late in a season, depending on the cultivar (Lewis, 1978; Kaiser & Wolstenholme, 1994; Requejo-Tapia, 1999; Woolf et al., 2009).

The main varieties that supply the domestic market in Brazil are: Geada, Quintal, Fortuna, Breda and Margarida. The main variety for export and/or industrialization is Hass (Yamanishi, 2011).

Table (1): Avocado: production of ten major producer countries in the world.

		Production (t)						
	Country	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
1	Mexico	1.107.140	1.264.141	1.316.104	1.467.837	1.520.695	1.644.226	1.889.354
2	Rep. Dominicana	275.569	295.080	290.011	387.546	428.301	526.438	601.349
3	Peru	184.370	212.857	215.000	288.387	349.317	367.110	455.394
4	Colombia	201.869	215.095	219.352	303.340	288.739	309.852	309.431
5	Indonesia	224.278	275.953	294.200	276.311	307.326	382.530	304.938
6	Brazil	152.187	160.376	159.903	157.482	156.699	180.652	195.492
7	Kenya	113 206	201.478	160.000	191.505	218.692	136.420	176.045
8	USA	149.300	238.544	245.000	175.226	179.124	203.209	172.630
9	Chile	330.000	368.568	160.000	164.750	160.000	146.204	137.365
10	China	105.400	108.500	110.000	112.000	116.000	117.938	128.601

Source: FAOSTAT (2018).

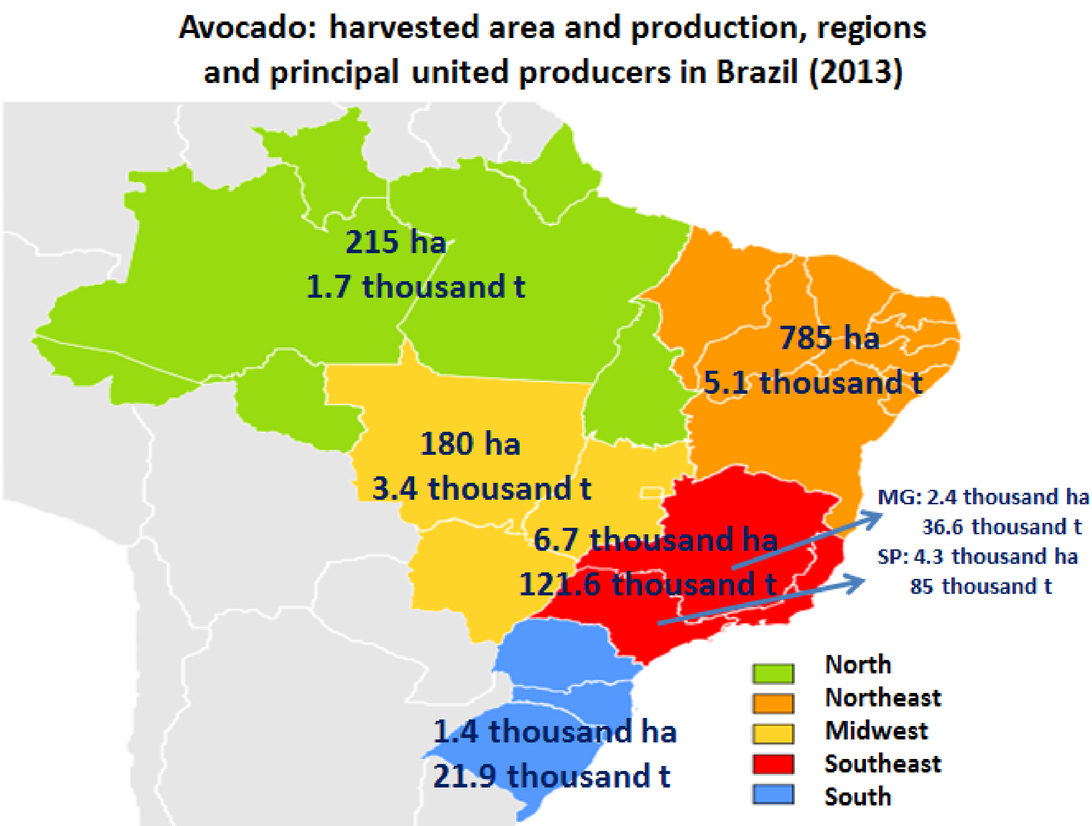


Figure1. **Avocado: harvested area and production, regions and principal united producers in Brazil. Source: AGRIANUAL, (2016)**

2.2.Chemical Composition of Avocado Oil:

2.2.1. Saponifiable Fraction: Fats and oils are constructed of building blocks called "triglycerides" (also known as triacylglycerols) resulting from the combination of one unit of glycerol and three units of fatty acids. They are insoluble in water but soluble in most organic solvents. They have lower densities than water, and may have consistencies at ambient temperature of solid, semi-solid, or clear liquid. When they are solid appearing at a normal room temperature, they are referred to as "fats," and when they are liquid at that temperature, they are called "oils."

2.2.1.1. Fatty Acid Composition

Atypical avocado oil is comprised mostly of monounsaturated fatty acids (74%), 11% polyunsaturated fatty acids and about 13% saturated (Arpaia *et al.*, 2006). These percentages vary slightly with cultivars and other factors but the oil is very similar to olive oil. It is this high level of monounsaturated fat, which gives the desirable effect of being "anticholesterol" as it prevents the formation of clots the major cause of coronary heart disease (Zarrabal*et a*l., 2014).

Table (2): Typical analysis of the Fatty Acid composition of Avocado Oil as compared to Olive Oil

Fatty Acids	Fatty Acids	Africa Oil Analysis (%)	New Zealand Oil Analysis (%)	
		Avocado Oil	**Avocado Oil**	**Olive Oil**
Palmitic Acid	C16:0	11.85	12.5-14.0	8.6-12.9
Palmitoleic Acid	C16:1	3.98	4.0-5.0	0.3-0.7
Stearic Acid	C18:0	0.87	0.2-0.4	2.1-2.8
Oleic Acid	C18:1	70.54	70-74	77-82.6
Linoleic Acid	C18:2	9.45	9.0-10.0	4.6-7.5
Linolenic Acid	C18:3	0.87	0.3-0.6	0.5-0.7
Arachidic Acid	C20:0	0.50	0.1	0.0-0.6
Gadoleic Acid	C20:1	-	0.1	0.0-1.4
Eliosenoic Acid	C20:1	0.39	-	-

(Eyres *et al.*, 2006; Human, 1987).

Table 2 lists results from two different countries and their analysis of avocado oil confirms the healthy composition of the oil in terms of fatty acid composition. It also indicates the comparability of avocado oil to olive oil due to very similar fatty acid composition. The analysis reported in table 2 was done using the Fatty Acid Methyl Ester (FAME) analysis on a Gas Chromatograph.

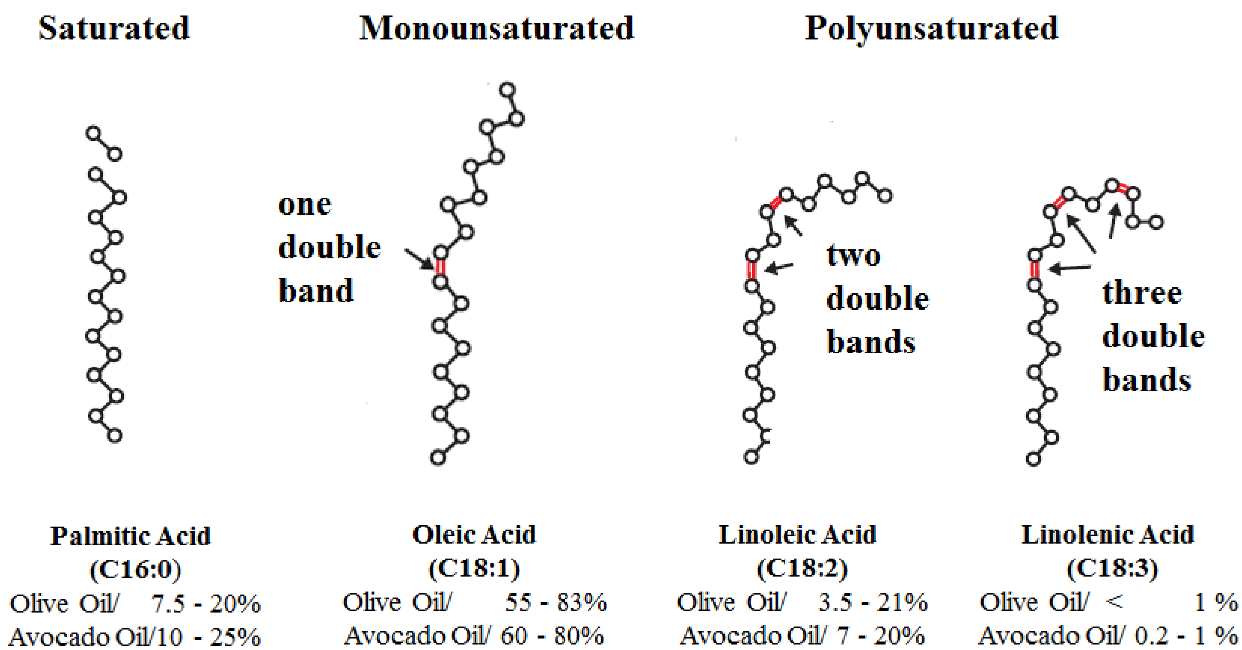


Figure 2. Forms of major fatty acids in olive oil and Avocado Oil.
Source: IOC, 2015; Wong et al., 2010

During Wong et al., 2010 in figure 2 avocado oil is a source high in monounsaturated fatty acids. avocado oil contains on average 60 to 80 % oleic acid, polyunsaturated fatty acids 7to 20% as linoleic acidand saturated fatty acid 10 to 25% as palmitic acid. depending on cultivar, growing region and time in the season. This compares favorably toolive oil which contains 55- 83 % oleic acid and from 3.5 to 21 % polyunsaturated fatty acids (linoleic acid) and from 7.5 to 20% Palmitic acid (IOC, 2015).The nutritional properties of olive oil, as a cholesterol-reducing food, are well known, and are shown by the low indexes of coronary diseases in Mediterranean countries, where consumption of this product is high (Andrikopolous, 1989). In fact, among people who live around the Mediterranean, heart disease is uncommon (Grundy, 1988). The high concentration of monounsaturated fatty acids in avocado suggests that a diet rich in avocado will have beneficial effects on blood lipids.

2.2.2. Unsaponifiable Matter (UM)

An important characteristic of this fruit is the high content of unsaponifiable matter (UM) 1 to 4% when compared with that of common edible oils (Turatti and Canto, 1985). Lozano et al., (1993) noted that the UM in crude oil of four cultivars of avocado was always higher in immature fruits than in mature fruits (15–40%*vs.* 4–

9%). The sterol content in the oil was always higher in immature (1.1–6.2%) than in mature (0.8–2.0%) fruits.

2.2.2.1. Sterols: The main constituent of this group is the β-sitosterol, comprising about 80% of the sterols. Other types of sterols also present are campesterol, stigmasterol, and cholesterol (law, 2000).Beta-sitosterol (a phytosterol) is one of the healthy plant compounds found to be most abundant in avocado. It is widely proven to be responsible for the non-absorption of the bad cholesterol (LDL) and maintaining the good HDL cholesterol in the intestine which then lowers total plasma cholesterol (Arpaia *et al.*, 2006). This compound was also reported by the British Medical Journal the Lancet to be very effective in offering relief to men above 50 years who suffered from benign prostatic hyperplasia resulting in significant improvements in urinary difficulties. These phytosterols have a similar chemical structure to cholesterol, differing only in their side chain lengths, and this structure similarity explains the ability of phytosterols to reduce cholesterol (Hicks and Moreau, 2001).

2.2.2.2.Tocopherols and Tocotrienols. Tocopherols and tocotrienols are important minor constituents of most vegetable fats. They serve as antioxidants to retard rancidity and as sources of the essential nutrient vitamin E. The common types of tocopherols and tocotrienols are alpha, beta, gamma, and delta.α – tocopherol is one powerful antioxidant, which neutralizes the free radicals produced under the normal metabolism of lipid compounds (Arpaia *et al.*, 2006).Tocopherols are commonly used as antioxidants (Murcia et al., 2001) because they give the hydrogen from the hydroxyl group to the peroxyl radical. They can also inhibit lipid peroxidation by sequestering the singlet oxygen (Kamal-Eldin and Appelqvist, 1996; Fukuzawa et al., 1998) and free radicals (Schuler, 1990).Tocopherols which occur naturally in most vegetable oils are partially removed during processing. The tocopherol content differed with the cultivars (10.2–25.0 mg/100 g UM), and the levels in the oil were higher in immature (20.1–45.6 mg/100 g oil) than in mature (5.7–10.3 mg/100 g oil) fruits (Lozano et al., 1993).

2.2.2.3. Chlorophyll & Carotenoids: Chlorophyll is a group of green colour pigments, carotenoids are the chemical compounds that reflect Yellow, orange and red colours. They are not soluble in water. Instead, they are soluble in fat. Crude and virgin oils have high amount of chlorophyll and carotenoids. The virgin oil with high chlorophyll content is highly desired by consumers due to the health benefits associated with the presence of these micronutrients. The high chlorophyll content makes the oil highly prone to oxidative effects upon exposure to light and for this reason must be packed in dark bottles. carotenoids like lutein are also present in high amounts while others like neoxanthin, violaxanthin, antheraxanthin are present in very minute amounts (Arpaia *et al*., 2006). Further processing and refining removes the chlorophyll and other pigments giving oil that is pale yellow in colour and also more stable (Botha, 2004; Eyres *et al*., 2006).

2.2.2.4. Mono- and Diglycerides. Mono- and diglycerides are mono- and diesters of fatty acids and glycerol.

2.2.2.5. Free Fatty Acids. As the name suggests, free fatty acids are the unattached fatty acids present in a fat. Some unrefined oils may contain as much as several percent free fatty acids. The levels of free fatty acids are reduced in the refining process.

2.2.2.6.Phosphatides. Phosphatides, also known as phospholipids, consist of an alcohol (usually glycerol) combined with fatty acids, and a phosphate ester. The majority of the phosphatides are removed from oil during the degumming and refining operations. Phosphatides are an important source of natural emulsifiers marketed as lecithin (Chairman et al., 2006).

2.3. Standards For Avocado Oil

The impacts of postharvest procedures, preprocessing treatments, extraction, and storage on the composition, quality, and sensory characteristics of avocado oil have been investigated over the last 10 years in New Zealand in collaboration with Australian and Californian research groups. Standards have been proposed for avocado oil, including extra virgin, virgin, and pure grades oil (Table 3). These standards have been recommended to ensure that avocado oil sold is of good quality in terms of standard quality indices, composition, and sensory properties. The standards are unique to avocado oil, where cold-pressed avocado oil is recovered by mechanical extraction at temperatures less than 50°C, without solvents; water and enzymes can be used. These standards are important, as the production and culinary consumption of cold-pressed avocado oil, with its light, distinctive flavor, is increasing worldwide.

TABLE 3. Proposed standards for avocado oil[a]				
	Extra virgin	**Virgin**	**Pure**	**blend**
General	Oil extracted from high- quality fruit (minimal levels of rots and physiological disorders). Extraction to be carried out using only mechanical extraction methods including presses, decanters, and screw presses at low temperatures (<50°C). Addition of water and processing aids (e.g., enzymes and talcum powder) is acceptable, but no chemical solvents can be used	Oil extracted from sound fruit with some rots or physiological disorders. Extraction to be carried out using only mechanical extraction methods including presses, decanters, and screw presses at low temperature (<50°C). Addition of water and processing aids (e.g., enzymes and talcum powder) is acceptable, but no chemical solvents can be used	Fruit quality not important. Decolorized and deodorized oil with low acidity. Low color, and bland flavor. Oil produced from good quality virgin avocado oil; may be just avocado oil or infused with natural herb or fruit flavors.	Avocado oil is excellent for blending and complements extra virgin olive, flaxseed, macadamia, and pumpkin seed oils. The specification and composition should match what is claimed on the label.
Organoleptic characteristics*b*				
Odor and taste	Characteristic avocado flavor and sensory assessment shows at least moderate (above 40 on a 100-point scale) levels of grassy and mushroom/butter with some smoky	Characteristic avocado flavor and sensory assessment shows at some (above 20 on a 100-point scale) levels of grassy and mushroom/butter with some smoky .	Bland or matches description of infused flavor, e.g., lemon, chili, rosemary, etc.	Dependent on the blend.
defects	Minimal to no defects such as painty and fishy notes below 20 and glue-like below 35 as a sensory panel average on a 100 point scale.	Low levels only of defects such as painty and fishy notes below 50 as a sensory panel average on a 100-point scale average on a 100-point scale	Low defects such as painty and fishy notes below 50 as a sensory panel average on a 100-point scale average on a 100-point scale.	Low defects such as painty and fishy notes below 50 as a sensory panel average on a 100-point scale average on a 100-point scale.
Color	Intense and attractive green	Green with potential yellow hue	Pale yellow	Dependent on the blend
Free fatty acid (% as oleic acid)	≤0.5%	0.8-1.0%	≤0.1%	As specified
Acid value	≤1	≤2.0	≤0.2	
Peroxide value (meq/kg oil)	≤4.0	<8.0	<0.5	
Smoke	≥250℃	≥200℃	≥250℃	

point				
Moisture	≤0.1%	≤0.1%	≤0.1%	
Fatty acid composition % (typical values)				
Palmitic acid (16:0)	10-25			
Palmitoleic acid (16:1)	2-8			
Stearic acid (18:0)	0.1-0.4			
Oleic acid (18:1)	60-80			
Linoleic acid (18:2)	7-20			
Linolenic acid (18:3)	0.2-1			
Antioxidants (mg/kg)				
Vitamin E	70-190			
Trace metals (mg/kg)				
Copper	≤0.05	≤0.05	≤0.05	≤0.05
*a*Reproduced from Woolf *et al., 2009* *b*These characteristics are measured with a trained sensory panel with a minimum of 15 hours of experience of tasting olive oil.				

2.4.Factors Influencing the Oil Content of Avocado

Avocado fruits with high oil content must be used in the production of oil. Various factors however are known to affect the oil content of fruits and they are:

Cultivar -Different cultivars vary in oil content upon maturity and only those with high oil content should be considered. Because the oil is contained in the pulp or flesh, cultivars with high proportion of flesh and minimum seed and peel should also be selected. Many studies have confirmed the Hass cultivar to be superior in quality with all the favorable attributes (Human,1987).

Maturity stage –The time at which the fruits of any given cultivar is harvested was noted by (Arpaia et al., 2006) to have great impact on the oil content of the fruits. Maturity is when the fruit is most suitable for human consumption and not for processing. Some cultivars mature early while others mature much later and understanding this becomes very important for choosing when to harvest. However, it is understood that when avocado fruits mature their moisture content lower while their oil increases and leaving the fruits on the trees much longer after maturity tend to increase oil content (Human, 1987).

Location and growth conditions - The same study of avocado postharvest quality by (Arpaia et al., 2006) also noted differences in oil content for the same cultivar due to different locations and growth conditions such as soil fertility. Sun exposed fruits were also found by (Woolf et al., 1999) to yield higher levels of oil than those fruits in the shade.

2.5. Uses of avocado oil:

Either crude or semi-refinedavocado oil can be used in pharmaceutical and cosmetic industries while refined avocado oil can be used in both cosmetics and food industries. Two examples of food oils are: salad oil and cooking oil (Tango and Turatti, 1992).

2.5.1. Food: The oil is highly applicable for food preparation and because of its high quality properties which is similar to olive oil, it provides another healthy alternative for consumers. It is marketed as healthy oil due to its high content of monounsaturated fat, presence of high vitamin content. The high sterol content in particular and its cholesterol lowering effect is what makes this oil a must for modern society. In many countries, avocado is consumed as a vegetable in the form of salads, with onions, cheese, salt and pepper as soup, or as a canned product (Teixeira et al., 1992). In Brazil, it is often used in sweet dishes, by mixing fresh avocado with cream, sugar in the form of shakes, and ice creams. avocado can also be used in savory dishes such as guacamole which common in Mexico (Oliveira et al., 2003).

2.5.2. Cosmetic Industry: Avocado oil is well known for its anti-bacterial, anti-wrinkle and healing properties. The multiple properties of avocado oil namely stability, emollience; skin penetration, softening and moisturizing, results in its wide applicability for cosmetic products. The high penetration ability of the oil makes it very successful in its use as a natural and effective beauty aid. Swisher (1988) mentioned that product information from the U.S Food and Drug Administration (1976) shows a total of 240 products containing avocado oil in concentrations ranging from 0.1 to 50%. Avocado oil is used in the formulation of cleansing creams, lipsticks, moisturizers, hair conditioners, suntan lotions, bath oils and make-up bases. Avocado oil demonstrated the highest rate of skin penetration (similar to lanolin). At present time, avocado oil is mostly used in the cosmetic industry as an ingredient in a range of products (Human, 1987). The high cost of the fruit itself makes the production of avocado oil an expensive product that can be paid only by the cosmetic or pharmaceutics industry.

2.5.3. Dietary supplement: One of the most exciting products produced by New Zealand based Olivado and Elysian Isle companies is the avocado oil capsule. It simply takes all the beneficial attributes found in avocado oil and seal it in a health capsule, which can be taken daily. It contains all the healthy micronutrients and vitamins available in the oil, which can help, fight bad cholesterol, inflammatory, osteoarthritis and prostate problems. Preliminary evidence suggests beneficial effects of fruit phenolics on reducing CVD risk by reducing oxidative and inflammatory stress, enhancing blood flow and arterial endothelial health, and inhibiting platelet aggregation to help maintain vascular health (Chong et al., 2010; Arts and Hollman, 2005; Ghosh and Scheepens, 2009; Victor et al., 2009).

Avocados have the highest fruit lipophilic antioxidant capacity, which may be one factor in helping to reduce serum lipid peroxidation and promoting vascular health (Wu et al., 2007). Avocados contain a moderate level of phenolic compounds contributing 60 mg and 140 mg gallic acid equivalents (GAE) per 30 g and one-half fruit, respectively. The avocado also has a total antioxidant capacity of 600 μmol Trolox Equilvalent (TE) per 30 g or 1350 μmol TE per one-half fruit (Wu et al., 2004; 2007). This places avocados in the mid-range of fruit phenolic levels. Avocados have the highest fruit lipophilic antioxidant capacity, which may be one factor in helping to reduce serum lipid peroxidation and promoting vascular health (Wu et al., 2007).several exploratory trials suggest that MUFA rich diets help protect against abdominal fat accumulation and diabetic health complications (Tentolouris et al., 2008; Paniagua et al., 2007a; 2007b).

2.5.4. Healthy Aging

2.5.4.1. DNA Damage Protection

Several clinical studies suggest that xanthophyll's, similar to those found in avocados, may have antioxidant and DNA protective effects with possible healthy aging protective effects(Yong et al., 2009).

2.5.4.2. Osteoarthritis

Osteoarthritis (OA) is characterized by progressive deterioration of joint cartilage and function with associated impairment, and this affects most people as they age or become overweight or obese (Dinubile, 2010; Helmick et al., 2008).Avocado and soy unsaponifiables (ASU) is a mixture of fatsoluble extracts in a ratio of about 1(avocado):2(soy). The major components of ASU are considered anti-inflammatory com-pounds with both antioxidant and analgesic activities (Dinubile,2010; Lipiello et al., 2008; Au et al., 2007; Henroitin et al.,2006; Berenbaum, 2004; Ernst, 2003; Blotman et al., 1997).

Clinical support for ASU in the management of hip and kneeOA comes from four randomized controlled trials (Lequesneet al., 2002; Appelboom et al., 2001; Maheu et al., 1998; Blotman et al., 1997) and one meta-analysis (Christensen et al.,2008). All studies used 300 mg per day. The clinical trials were generally positive with three providing OA support and one study showing no joint cartilage improvement compared to placebo.

2.5.4.3. Eye Health

Lutein and zeaxanthin are two types of carotenoids. which are yellow to red pigments found widely in vegetables and other plants.In nature, lutein and zeaxanthin appear to absorb excess light energy to prevent damage to plants from too much sunlight, especially from high-energy light rays called blue light.

In addition to being found in many green leafy plants and colorful fruits and vegetables, lutein and zeaxanthin are found in high concentrations in the macula of the human eye, giving the macula its yellowish color. In fact, the macula also is called the "macula lutea" (from the Latin macula, meaning "spot," and lutea, meaning "yellow").Recent research has discovered a third carotenoid in the macula. Called meso-zeaxanthin, this pigment is not found in food sources and appears to be created in the retina from ingested lutein. Two studies published in Investigative Ophthalmology and Visual Science found that eyes with greater levels of macular pigments were less likely to have or develop macular degeneration (Nolan et al., 2013).Lutein and zeaxanthin are selectively taken up into the macula of the eye (the portion of the eye where light is focused on the lens) (Capentier et al., 2009).Avocados may contribute to eye health since they contain a combination of MUFA andlutein/zeaxanthin and help improve carotenoid absorption from other fruits and vegetables (Unlu et al., 2005). Avocados contain 185 μg of lutein/zeaxanthin per one-half;Hass fruit; 68g, which is expected to be more highly bioavailable than most other fruit and vegetable sources(USDA, 2011).

2.5.4.4. Skin Health

Skin often shows the first visible indication of aging. Topical application or consumption of some fruits and vegetables or their extracts such as avocado has been recommended for skin health (Roberts et al., 2009; Morganti et al., 2002; 2004).

2.5.4.5. Cancer

Avocados contain a number of bioactive phytochemicals including carotenoids, terpenoids, D-mannoheptulose, persenone A and B, phenols, and glutathione that have been reported to have anti-carcinogenic properties (Ding et al., 2009; Jones et al., 1992; Ames, 1983).

2.5.6. Biodiesel

All vegetable oils may be processed into biodiesel. In this way, the vegetable oils of the following species can be the raw material for biodiesel production: peanut kernels, palm pulp, sunflower seed, castor seed, passion fruit seed, avocado pulp, among many others vegetables in the form of seeds, such as almonds or the pulps (GÓES, 2006).

According to Arima et al.(1985)avocado oil yield obtained per hectare may be 5.5 times greater than the soybean and 4 times higher than peanut. The avocado production operating costs are in a position intermediate between soybean and peanut. Some other vegetable varieties have relatively high content of fatty matter.

Avocado can be a new alternative for biodiesel production, according to a study by researchers at the Universidade Estadual Paulista (UNESP). They found that avocado has the advantage as compared to other oil studied or used for the production of biofuels, such as soybean oil. The reason is that the same result is possible to extract the two main raw materials of biodiesel: oil from (pulp) and ethyl alcohol from (the seed).

Among of all methods studied by the group led by Menezes for oil extraction, the best result was obtained with drying. A rotary furnace with hot air was developed and, after drying, the pulp was ground and placed in the press, then the solvent suspension process was applied. From that moment, it was transferred to a basket centrifuge, developed by the group. "With the centrifugal force, the pulp turns very dry and the yield improves," noted Menezes, 2009.

The fruit takes a lot of energy in the drying process. However, researchers believe that the productivity and the presence of alcohol and oil from the same source offset the expense.

Improvisations - To get to the final product, Menezes had to go beyond chemistry. The first challenge was to extract, “using enzyme, acid hydrolysis and alkaline, to discover that dehydration was the best option," he explains. But dehydrating the fruit pulp was not easy. In traditional ovens, he ended up burning everything. Until the day he adapted an old washing machine with front cover to turn it into a rotary kiln.

Economic viability: According to the study done in UNESP, avocado oil biodiesel characteristics are quite similar to those seen with soy biodiesel, except for the color, which is green for avocado and yellow for soybeans. But this would not affect the quality.

The cost of biodiesel is still high. Soybean oil is produced at a cost of 1.20 R $/liter. Anhydrous alcohol is purchased to add to 0.74 R $/liter. The avocado has the advantage of offering the substance at a lower cost than soybeans, according to Menezes.

According Menezes, avocado oil content ranges from 5% to 30%. However, samples collected in Bauru (SP) had, at most, 16% oil content. "This index is similar to soybean oil content in the same region, which is 18%," as he compared.

"Theoretically, it is possible to extract 2200-2800 liters of avocado oil per hectare" he said. This number is considered high when compared to the extraction of other oils, for example: soybean (440 to 550 liters / hectare) castor bean(740 to 1000 liters / hectare), sunflower (720 to 940 liters / hectare) and cotton (280 to 340 liters / hectare).

Avocado core has 20% starch. Based on this percentage, it is estimated that one can extract 74 liters of alcohol per ton of avocado pits. Value close to that of sugarcane, which enables the extraction of 85 liters per tone, while cassava provides 104 liters per tone (Agência FAPESP, 2009).

2.6. Processing of Avocado Oil

In order to produce a high quality product it requires the availability of high quality raw materials. For this reason it is imperative that the fruits used are of high grade in particular and must contain high proportion of pulp, nevertheless, have high oil content, free of diseases and must not be physically damaged as evident in pulp discoloration and/or off-flavors. The fruits must be carefully matured and ripened to allow maximum oil development before they are used.

Various methods have been used in the extraction of oil from avocado fruits. The methods vary in their degree of effectiveness for oil extraction and also subsequent effect on the resultant oil quality. Heating and chemical extraction have been the traditional methods used and now a new method has been developed in New Zealand which allows oil to be expressed from the fruits with very minimal processing. Human (1987) lists various methods used in the past for the extraction of avocado oil involving steam pressure, hydraulic pressing, solvent extraction, centrifugation, freeze-drying, rendering process and the use of a tube press plant. However the specific application of the oil should determine the process and method chosen. Only the most practical and suitable methods are discussed below.

Pre-Process Treatment

Avocado fruits destined for oil production must be firstly inspected for physical damage and other abnormalities. They are then washed before being processed to remove the skin and seed. The means used for washing, de-stoning and de-skinning of the fruits vary but all processes involve this first critical step.

Rendering process **-** This method involves heating of the avocado pulp in avocado oil. The moisture evaporates off leaving the oil and the dry matter behind. The oil can then be decanted off while the remaining slurry is subjected to hydraulic pressure to press out more or the remaining oil. It is reported that laboratory experiments resulted in 94% recovery of the oil using this method (Human, 1987).

Tube Press Plant– This consist of either one or two tubes. The tubes are filled with avocado pulp and then subjected to hydraulic pressure to press out the oil through perforations within the inner tubes. This method aims at extracting out most of the oil with minimal damage to the oil quality (Human, 1987).

Solvent Extraction– This is one of the traditional methods commonly used. Various solvents can be used and organic solvents have been mostly utilized. Botha (2004) reported experimental solvent extractions using hexane and a Soxhlet extractor on a 10g dried sample for 8 hours. Subsequent removal of the solvent followed by vacuum evaporation and drying until constant mass. The resultant oil has a high chlorophyll content meaning the chlorophyll is co-extracted along with the oil. Chlorophyll levels as high as 192.9 ppm were reported by Werman & Neeman (1987) to have been extracted in the laboratory by ethanol extraction.
Human (1987) reported this method to have the highest yield but industrial equipment is very expensive to install and the highly flammable solvents used are very dangerous. The recovery and total removal of the solvent is also an issue, which requires a highly sophisticated plant. Thus because the raw material is an expensive fruit and the oil yield is at maximum around 22% of the whole fruit it is not economical to extract avocado oil in this way. Xiao *et al.*,(2006) reported that

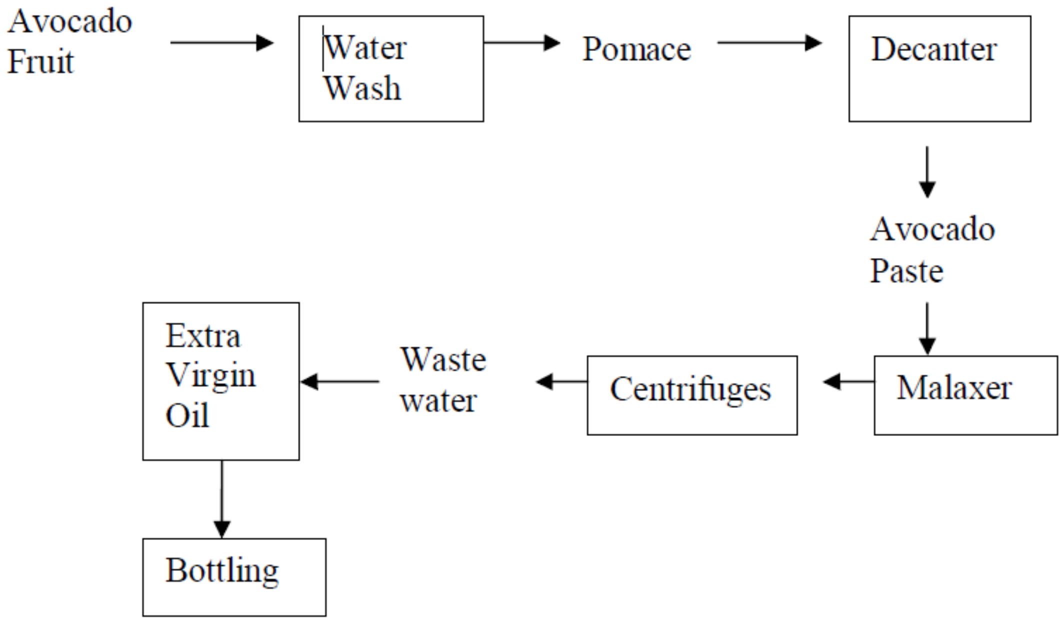
method does have other disadvantages like loss of volatile compounds, long extraction times, toxic solvent residues and degradation of valuable oil compounds.

Centrifugation– After the pre-processing treatment, the fruits are fed into a mill where it takes the form of a guacamole. The malaxation takes up to several hours until the release of a fine emulsion of oil. The paste is then fed to a centrifugal decanter where the oil is separated from the guacamole.
Werman & Neeman (1987) and Bizimana *et al.* (1993) reported that extraction of oil was most efficient using centrifugal force 12,300 xg, a 5:1 water to avocado ratio, temperature 75ºC, with a pH 5.5 and a 5% concentration of either NaCl, $CaCO_3$ or $CaSO_4$.

Cold Press- as it is in figure (3) This relatively new method of extracting oil makes use of the modern Alfa Laval centrifugal extraction method so it is a variation of the above method. The fruit flesh is firstly macerated by high speed grinders before the mixture is mixed in malaxers. When this process is complete, a three phase decanter then separates the mixture into oil, water and solids before polishing takes place with a multi-cone centrifuge. Extra virgin oil is produced after the first press. The extraction efficiency is dependant on such things like pH, centrifugation rate, salt, mixing temperature (<50ºC) and duration of pressing (Eyres *et al.*, 2006; Sionek, 1997). At no time along the whole process is the oil subjected to light or oxygen as this has a deteriorating effect on the quality of the oil. The extraction rates vary from 10-22% of the whole fruit and tend to vary during the season.

The production of cold pressed oil requires little investment and the process itself is simple. However, the process has inefficiencies in that around 6-15% of the oil remains in the pressed residue (Uzytku & Higieny, 1997).

Cold Press Extraction Process



(Wong *et al*., 2006)

Figure (3): cold press extraction process

The above method involves low temperatures and minimal processing and as a result, the oil retains all its natural flavour, nutrients and healthy properties. Thus, the resultant oil is of high quality and is considered a "virgin oil" because of very minimal processes involved. This virgin oil however has high chlorophyll content and is thus more unstable. This makes packaging in dark bottles or tins and total avoidance of oxygen a must for a longer shelf life for the product. This oil must be stored in a dark, cool cupboard where the temperature never rises above 30^{o}C, but should never be refrigerated or it will solidify. Virgin oil has a shelf life of two years only if stored correctly. More stable refined cold processed oil can also be produced after it has undergone further processes of refining, bleaching and deodorization (RBD).

Supercritical CO2– Botha (2004) reported extraction results for avocado oil extracted using supercritical carbon dioxide under four different extraction conditions. The use of supercritical fluid is proven to be a cost-effective technique for laboratory scale while large scale units still require experimentation for accurate economic evaluations. This method has advantages such as low operating temperatures, shorter extraction periods, high selectivity in the extraction of compounds and no undesirable solvent residue (Xiao *et a*l., 2006). It also uses a safe, readily available gas.

2.7. Refining of Avocado Oil

Virgin and crude oil can be further processed when it does not meet the virgin oil quality standards. The crude oil with high chlorophyll content is a dark green transparent oil with brown or yellow tints. This highly unstable oil could be refined using the following steps.

Bleaching

Bleaching removes colour pigments like chlorophyll and lutein using acidified activated earth at an elevated temperature. This is followed by filtration and a spectrophotometer may be used to monitor the bleaching process by measuring the optical density of the oil. Losses as high as 5% may be encountered here.

Deodorising

Deodorising is required to remove objectionable flavours/odours, which may develop during bleaching by using steam distillation. This is done under vacuum at elevated temperatures. Again losses as high as 7% may be incurred here.

Winterising

The presence of high melting components in the oil makes it cloudy at low temperatures. These components tend to crystallize at low temperatures and must be removed. Winterising aid like oxystearin is used to help the formation of larger crystals, which could then be removed via decanting and filtration.

Alkali Refining

The refining process involves the use of a strong alkali (eg. NaOH) to remove free fatty acids and peroxides. Both compounds tend to reduce the shelf life of the oil and also give it a rancid smell. This tedious process may result in losses as high as 7% - 8% (Human, 1987; Eyres *et al*., 2006). Nicolisi & Orthoefer (2004) reported that a patent (6, 197, 357) was awarded for the replacement of NaOH with $Na2CO_3$ or $NaHCO_3$ which resulted in a refining process that retained more than 85% of the phenolic compounds. These healthy compounds are usually lost with the unsaponifiable fraction of the oil during the refining process when NaOH is used.

Another method for refining is by heating the oil under vacuum at elevated temperatures, and sparged with live steam causing the free fatty acids to be distilled off refined oil is pale yellow, bland, and highly stable. It is highly suitable for general purpose cooking because of its high smoke point and has a healthy fatty acid composition. It is most suited for barbeques as most common vegetable oil polymerize and oxidize readily on hot surfaces.

2.8. Effect of Processing on the Quality of Oil

The phenolic compounds which are well publicized for their health benefits are lost or destroyed by most processing methods. Extra virgin oil loses its highly beneficial micronutrients during the refining process and the cholesterol lowering and anti-oxidant properties are significantly reduced as a result (Nicolisi& Orthoefer, 2004). The colour or chlorophyll content of the oil is noticeably lower than that of virgin oil due to the removal of these plant compounds (Eyres *et al*., 2006).

The cold pressing and supercritical fluid methods appear to be the most suitable and beneficial methods to extract the avocado oil. Both utilise low temperatures, which help retain the healthful composition of the oil, involves minimal processing and requires less capital investment.

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3. HYPOTHESES

- Is it better to extract avocado oil immediately after harvest, as the olives, or wait?
- Are the content of avocado dry matter and oil going to change after harvest during storage?
- What is the fatty acid composition of avocado oil for *Hass*, *Breda* and *Margarida*cultivars at six storage period including rejected fruits due to long storage period at room temperature (25±5ºC)?
- Is it possible to make use of rejected avocado fruits?
- Is it possible to extract avocado oil in a new methodology unlike the methods used commercially in mean of without usage of (enzyme, supercritical CO2, acid hydrolysis, alkaline, solvent, heating, or add water)?
- Are there differences in the quantitative and qualitative characteristics of the oil of different varieties of avocado grown in Brazil During different harvesting time to determine the proper harvesting time to extract oil?
- Are selected cultivars optimal to extract oil?

4. JUSTIFICATION:

Avocado oil is definitely a more productive use of the fruit. It can act as a local substitute for imported vegetable oil and as an export item both as a cooking oil and as a raw material for cosmetic and pharmaceutical products. The production of avocado oil is therefore an excellent option for making value added products from this wasted produce which has high economic value due to its highly healthy composition, and to extract the two main raw materials of biodiesel: oil (pulp) and ethanol (biofuel) (the seed).

5. EXPERIMENT I

CHANGES IN DRY MATTER, OIL CONTENT AND FATTY ACID OF AVOCADO DURING STORAGE

Abstract

Changes in dry matter and oil contents in the fruit of three avocado (*Persea americana* Mill.) cultivars: 'Hass', 'Breda' and 'Margarida' were measured during the storage period. In mid-October 2014, avocado fruits were harvested according to the Tsuge Farm picking standard in Rio Paranaíba city, Minas Gerais State, Brazil. Samples were kept at room temperature (25±5ºC) for 22 days and content of dry matter and oil were measured six times at different time intervals. The dry matter content in 'Hass' and 'Breda' increased from 34.1 to 45.2% and from 29.6 to 32.9%, respectively during storage. Oil content increased significantly from 20.5 to 35.7% in 'Hass' and from 14.3 to 25.7% in 'Breda'. On the other hand, oil content in 'Margarida' fluctuated during storage. Oleic (51.2/60.1/55.9%), palmitic (21.8/22.8/26.9%), linoleic (13.6/9.7/13.2%) and palmitoleic (13.1/7.0/3.0%) acids were the major fatty acids observed in 'Hass', 'Breda' and 'Margarida', respectively and their content significantly changed during storage.

Keywords: avocado, 'Hass', 'Breda', 'Margarida', fatty acid composition, ripening

5.1. INTRODUCTION

Avocado (*Persea americana* Mill.) fruit is very much appreciated, and it occupies a prominent place in the market due to its high nutritional value, especially fibers and lipids. Vegetable oils are the major source of edible lipids, which are consumed all over the world. They are extracted either from the endosperm of the oil seeds or from the pericarp of oil fruits, mainly palm and olive. Another important oil fruit is avocado. The avocado tree is a fruit plant that originated in the Americas, especially Mexico and Central America, belonging to the *Lauraceae* family (Maranca, 1980; Koller, 1992).

It is one of the most productive plants per unit of cultivated area. A large number of avocado cultivars are found in different regions of Brazil, whose fruits have varied chemical composition, especially in terms of levels of lipids in the pulp. Fruits with high levels of lipids in the pulp can be important raw material for oil extraction (Teixeira et al., 1991).

Mexico is the country that leads the production of avocado in the world; Brazil is the third largest world producer. However, the state of São Paulo is the largest domestic producer, accounting for more than 50% of the production. The state of Minas Gerais is the second, followed by Paraná and Espírito Santo (Agrianual, 2009).

Avocados are nutrient-dense fruits, high in unsaturated fats, fiber, niacin, folate, lutein, potassium, iron, and vitamins B6, C, E, and K (USDA-ARS, 2008). The oil quality is beneficial to cardiovascular health, with about 65 to 75% monounsaturated fatty acids (oleic and palmitoleic) and 10 to 15% polyunsaturated fatty acids (linoleic) (Ozdemir and Topuz, 2004). Avocado cultivars and maturity vary widely in fruit oil content, from 5 to >30% (Woolf et al., 2004).

Avocado oil content is highly correlated to fruit dry matter content (Lee et al., 1983). As fruit matures, the percentage of dry matter increases, as does the oil content; however, there is cultivar-to-cultivar variation in this relationship. The percentage of dry matter content has become the predominant maturity index for avocado harvesting, but it must be cultivar-specific (Bower and Cutting, 1988; Hofman et al., 2013; Lee et al., 1983). California, Australia, and Brazil also use the percentage of dry matter content as an indirect measure to determine oil content, and hence maturity, for different cultivars (Lee and Coggins, 1982; Woolf et al., 2004).

Various factors are known to affect the oil content of fruits including; cultivar, maturity stage, location and growth conditions.

Identification of horticultural maturity is difficult for many fruits, especially avocado, because maturation is not accompanied by changes in external appearance. Moreover, mature avocado fruit do not ripen on the tree, but soften several days after being picked (Cox et al., 2004).

The objective of this study was to analysis the effect of the storage periods on the contents of avocado; such as dry matter, oil content and fatty acids. The findings of this work will help the farmer and industrialist to understand the accurate time of fruit storage and oil extraction.

5.2. MATERIALS AND METHODS

5.2.1. MATERIALS

This investigation was carried out at Tsuge Farm,in Rio Paranaíba, in Minas Gerais State (MG), Brazil. The located at Lat. 19° 25′ 33″S ; Long. 46° 15′ 37″ W. Altitude (11080m over sea level). By Köppen's classification the climate of the region is Aw type in transition to Cwb, with average annual minimum temperature of 11.0 ° C. The mean annual temperature (MAT) averaged 21.1°C, mean annual maximum temperature of 22.3 ° C and total annual precipitation of 2713.65 mm. (PIRES; YAMANISH, 2014).Mature avocado trees on ten to twelve years old of three cultivars; Hass, Breda and Margarida. The trees were spaced 8 m X 6 m; not irrigated and received routine horticultural care (standard pest, disease &fertilizer programs). Avocados are harvested by hand from the trees. The mean yield (17.5/ 30/ 35)tone of fruit per hectare for Hass, Breda and Margarida cultivars respectively.

Three avocado cultivars: ‘Hass’, ‘Breda’ and ‘Margarida’ (*Persea americana* Mill.) were used in this experiment. Mature avocado fruits were harvested on October 17th, 2014 after 12 months from flowering, and stored at room temperature (25±5°C) for 22 days. During that time, analyses were carried out on the 5th, 8th, 12th, 15th, 19th and 22nd days of the storage period; the seed of the fruits were removed before analysis. All the experiments were conducted in triplicates.

5.2.2. METHODS

5.2.2.1. Dry matter contents

Dry matter content was determined by drying the samples at 40°C (5-6 days) to a constant mass. And Fresh weight of fruit was determined.

5.2.2.2. Oil content

Dry pulp oil content was determined by Soxhlet extraction, using hexane as described by IUPAC (1979) method 1.122. Oil content was expressed on a fresh weight basis.

5.2.2.3. Fatty acid composition of the extracted oil

Fatty acids were transformed into methyl esters (FAME) according to the method described by IOC (2001) and were determined using gas chromatograph: Shimadzu GCMS-QP2010 equipped with a flame ionization detector (FID).

5.2.2.4. Statistical analysis

The obtained data were statistically analyzed. Analysis of variance and Duncan multiple range test were performed using SAS to evaluate the significance of differences between values at the level of $P<0.05$. RCB design was used for statistical analysis.

5.3. RESULTS AND DISCUSSION

Table 4 shows that the dry matter in 'Hass' was increased during storage period (6 periods) from 34.1% in the first time of storage to 45.2%. That increase was at a rate of 32.4% over 22 days of storage. In a similar way, the oil content increased from 20.5 to 35.7% in the fresh pulp. This increase was at a rate of 42.6%.

Table 4. Fruit weight and changes in the oil content and dry matter of 'Hass' at six storage periods.

	Storage period (days)					
	5th	8th	12th	15th	19th	22nd
Fruit weight (g)	255a	265a	270a	230a	170b	210ab
Oil content (%)	20.5a	21.06a	23.04a	30.63b	32.35b	35.72c
Dry matter (%)	34.12a	33.33a	32.18a	35.62b	37.93c	45.16d

Means within a row followed by the same letter were not significantly different (P<0.05 by Duncan's multiple range test).

Similar results were found in 'Breda' (Table 5), the dry matter increased from 29.6 to 32.9% at a rate of 11.1%. In a similar way, oil content was increased from 14.3 to 25.7% in fresh pulp, at an increase rate of 79.6%. As it can be seen in Table 4, changes in oil content were parallel to changes in dry matter for both 'Hass' and 'Breda'. These results are consistent with Lee et al. (1983).

Table 5. Fruit weight and changes in the oil content and dry matter of 'Breda' at six storage periods.

	Storage period (days)					
	5th	8th	12th	15th	19th	22nd
Fruit weight (g)	510a	520a	500a	480a	435b	455b
Oil content (%)	14.31a	17.35b	19.14b	19.99b	23.66c	25.71d
Dry matter (%)	29.63a	27.63a	31.25b	33.77b	33.33b	32.88b

Means within a row followed by the same letter were not significantly different (P<0.05 by Duncan's multiple range test).

In the case of 'Margarida', Table 6 show that more oil content was found with less fruit weight. Moreover, the percentage of dry matter in 'Margarida' was: 22.0, 21.4, 25.8, 23.3, 19.7 and 17.4% during the six-storage period. Fruit oil content changed irregularly (increase and decrease) during storage period.

Table 6. Fruit weight and changes in the oil content and dry matter of 'Margarida' at six storage periods.

	Storage period (days)					
	5th	8th	12th	15th	19th	22nd
Fruit weight (g)	565a	655bd	575a	540a	690d	635b
Oil content (%)	15.21ac	8.79b	16.85c	15.09a	9.32b	8.24b
Dry matter (%)	21.98a	21.43a	25.77b	23.33c	19.67d	17.43e

Means within a row followed by the same letter were not significantly different (P<0.05 by Duncan's multiple range test).

The fatty acids composition of avocado oils of 'Hass', 'Breda' and 'Margarida' during six storage periods is shown in Table 7. Oleic acid was the principal fatty acid in all oils during the six storage periods (53.3-49.9/60.1-59.4/55.9-45.3%), followed by palmitic (22.1-19.3/23.2-19.9/27.0-22.4%), linolitic (13.6-17.7/9.7-14.6/13.2-26.4%), and palmitoleic (13.1-10.0/7.3-4.6/3.0-2.1%) acids were found to be major fatty acids, respectively, in avocado oil of 'Hass', 'Breda' and 'Margarida'. The fatty acid composition of avocado oil depends upon the cultivar, stage of ripening, the geographical growth location and different sample processing (Ahmed and Barmore, 1980; Bora et al., 2001; Moreno et al., 2003). These main fatty acids compositions agreed with previous studies (Jorge, 2014; Haiyan et al., 2007; Moreno et al., 2003; Yanty et al., 2013). Stearic acid was also determined to be present in small amounts in some periods and not present in other storage periods in 'Hass' and 'Margarida', while it was not presented in all periods of storage in 'Breda'. As well as linolenic acid, this was also determined to be present in small amounts in some storage periods and was not presented in other storage periods in all studied cultivars. Based on the linoleic and linolenic acid contents in the oils of the three avocado cultivars, it can be concluded that these cultivars are rich sources of these ω-3 and ω-6 fatty acids, which could contribute towards the daily needs of an adult, which is around 0.8 to 4.0 g (World Health Organization & Food and Agriculture Organization , 2003). Other researchers (Brasil, 1971; Bora et al., 2001; Frega et al ., 1990; Martinez Nieto et al ., 1988; Moreno et al ., 2003; Ratovohery et al ., 1988; Salgado et al ., 2008; Tango et al ., 1972, 2004; Villa-Rodríguez et al., 2011; Dreher and Davenport, 2013) have also reported oleic acid as the major fatty acid, followed by palmitic, linolenic, and palmitoleic acids in the pulp of 'Collinson', 'Barker', 'Fortuna', 'Lula', 'Bacon', 'Fuerte', 'Zutano', 'Hass', and 'Margarida' avocado fruit.

Table 7. The fatty acid composition expressed as % of avocado oil (‘Hass’, ‘Breda’, ‘Margarida’) at six storage periods.

Fatty acids	cultivars	Storage period (days)						Average
		5th	8th	12th	15th	19th	22nd	
Palimitic acid (C16:0)	Hass	21.8a	22.1a	19.28b	20.9c	21.78a	21.67a	21.26
	Breda	22.8a	20.26b	19.91b	20.07b	23.20c	20.18b	21.07
	Margarida	26.9a	23.99b	26.99a	26.99a	22.37c	24.49d	25.29
Palmitoleic acid (C16:1)	Hass	13.10a	11.30b	10.01c	10.22c	12.87a	12.49a	11.67
	Breda	7.00a	5.92b	4.55c	5.09c	7.33a	4.61c	5.75
	Margarida	3a	2.99a	2.3b	2.59ab	2.1b	2.44ab	2.57
Stearic acid (C18:0)	Hass	0.18a	nde	nde	nde	0.06b	0.28c	0.09
	Breda	nd	nd	nd	nd	nd	nd	0.00
	Margarida	1a	ndb	ndb	ndb	ndb	ndb	0.17
Oleic acid (C18:1)	Hass	51.2a	50.40ac	53.33b	51.17a	49.88cd	49.22d	50.87
	Breda	60.10ab	59.87ab	60.95a	61.07a	59.37b	60.87a	60.37
	Margarida	55.9a	53.49b	51.53c	50.33d	47.28e	45.26f	50.63
Linoleic acid (C18:2)	Hass	13.60a	16.19b	17.39c	17.71c	15.26d	15.99d	16.02
	Breda	9.70a	14.44b	14.58b	13.77b	9.94a	14.34b	12.80
	Margarida	13.2a	19.24b	19.18b	22.84c	26.39d	26.19d	21.17
Linolenic acid (C18:3)	Hass	0.12a	ndc	ndc	ndc	0.14a	0.33b	0.10
	Breda	0.40a	ndc	ndc	ndc	0.15b	ndc	0.09
	Margarida	nde	0.28a	nde	nde	1.86b	1.61c	0.63

The means followed by the different letters in the same row are significantly different ($p<0.05$ by Duncan’s multiple range test); nd: not detected.

During six storage periods within 22 days, the percentage of some fatty acids showed a significant change. In contrast, there were no significant differences in the percentage of other fatty acids. The highest level of oleic is owned by ‘Breda’ (61.1-59.4%) followed by ‘Margarida’ (55.9-45.3%) and ‘Hass’ (53.3-49.2%). While, the highest level of palmitic is owned by ‘Margarida’ (27.0-23.0%) followed by ‘Breda’ (23.2-19.9%) and ‘Hass’ (22.1-19.3%).

No clear trend was found for most of the fatty acids. There was a rapid increase in the percentage of linoleic acid in ‘Hass’ from 13.6% in 5th to 17.7% in 15th at the maximum increase rate of 30.2%, and then a decrease was observed in 19th to 15.3%, then a slight increase at the rate of 4.8% in the last storage period. In contrast, palmitoleic acid decreased from 13.1% in 5th to 10.0% in 12th, and then a slight increase was observed in 15th at a rate of 2.1% followed by more increase in 19th at a rate of (25.9%). The maximum value of oleic acid was at 53.3% in 12th, while the minimum at 49.2% in the last period of storage. There was also an irregular decrease and increase in the percentage of palmitic acid during six storage periods.

In ‘Breda’ palmitoleic and linoleic acid in particular, showed significant decrease and increase during storage period when compared with its initial value. The percentage of palmitoleic acid was decreased by 34.1% from first to the last period of storage for 22 days; in contrast the percentage of linoleic acid increased by

47.8% from first to the last period of storage. While oleic acid showed a slight changes during storage period.

Oleic acid was the only fatty acid which decreased steadily from the first to the last storage period of ‘Margarida’, with percentages ranging from 55.9 to 45.3% at a decrease rate of 19.0%, while linoleic acid showed an opposite trend with an 98.4% increment from the first period to the last period of storage.

The total values of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated (PUFA), and unsaturated fatty acids (UFA) in addition to the MUFA/SFA, PUFA/SFA, and UFA/SFA ratios during storage for three cultivars are presented in Table 8. Avocado oil samples had high amounts of total unsaturated fatty acids during through storage period (22 days) i.e., ‘Hass’ (77.9-80.7%), ‘Breda’ (76.8-80.2%) and ‘Margarida’ (72.1-77.6%), respectively. ‘Breda’ and ‘Hass’ are found to be healthier than ‘Margarida’, as they had the highest MUFA:SFA ratio, (3.3-2.9/3.3-2.8) in ‘Breda’ and ‘Hass’, respectively while (2.4-2.0) in ‘Margarida’.

Table 8. Saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), unsaturated fatty acids (UFA) profile (% of total fatty acids); MUFA/SFA, PUFA/SFA, and UFA/SFA mean value during of six storage periods from avocado fruit ‘Hass’, ‘Breda’, ‘Margarida’.

Cultivars	Fatty acids	Storage period (days) 5th	8th	12th	15th	19th	22nd	average
Hass	SFA	21.98	22.1	19.28	20.9	21.84	21.95	21.34
	MUFA	64.3	61.7	63.34	61.39	62.75	61.71	62.53
	PUFA	13.72	16.19	17.39	17.71	15.4	16.32	16.12
	UFA	78.02	77.89	80.73	79.1	78.15	78.03	78.65
	MUFAf/SFA	2.93	2.79	3.29	2.94	2.87	2.81	2.94
	PUFA/SFA	0.62	0.73	0.90	0.85	0.71	0.74	0.76
	UFA/SFA	3.55	3.52	4.19	3.78	3.58	3.55	3.70
Breda	SFA	22.8	20.26	19.91	20.07	23.2	20.18	21.07
	MUFA	67.1	65.79	65.5	66.16	66.7	65.48	66.12
	PUFA	10.1	14.44	14.58	13.77	10.09	14.34	12.89
	UFA	77.2	80.23	80.08	79.93	76.79	79.82	79.01
	MUFAf/SFA	2.94	3.25	3.29	3.30	2.88	3.24	3.15
	PUFA/SFA	0.44	0.71	0.73	0.69	0.43	0.71	0.62
	UFA/SFA	3.39	3.96	4.02	3.98	3.31	3.96	3.77
Margarida	SFA	27.9	23.99	26.99	26.99	22.37	24.49	25.46
	MUFA	58.9	56.48	53.83	52.92	49.38	47.7	53.20
	PUFA	13.2	19.52	19.18	22.84	28.25	27.8	21.80
	UFA	72.1	76	73.01	75.76	77.63	75.5	75.00
	MUFAf/SFA	2.11	2.35	1.99	1.96	2.21	1.95	2.10
	PUFA/SFA	0.47	0.81	0.71	0.85	1.26	1.14	0.87
	UFA/SFA	2.58	3.17	2.71	2.81	3.47	3.08	2.97

SFA:(C16:0+C18:0); MUSF:(C16:1+C18:1); PUFA:(C18:2+C18:3); UFA: (C16:1+C18:1+C18:2+C18:3)

Moreover, and from Tables 4 and 5, in general, it was noticed that there is no clear trend for fatty acid contents during storage periods. Also, there was no relation between molecular weight and degree of unsaturation, and the variations in these fatty acid contents with different storage periods, from different cultivars.

5.4. CONCLUSIONS

The highest value for dry matter and oil content was observed in 'Hass' followed by 'Breda', which make these cultivars more suitable for oil extraction, while the value was significantly lower in 'Margarida', although it is one of the most productive cultivars per unit area. Dry matter, oil content and the fatty acid composition of the oil changed differently with the avocado cultivars and storage period. The data is important to decide the proper storage period to extract oil with high quality and quantity.

5.5. ACKNOWLEDGEMENT

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6. EXPERIMENT-II

A NEW METHODOLOGY FOR EXTRA-VIRGIN AVOCADO OIL EXTRACTION *VERSUS* TRADITIONAL PRODUCTION METHODS

Abstract

Avocado (*Persea americana Mill*) is an oil-rich highly nutritious fruit. The pulp contains up to 30% of the oil (based on fresh weight). The avocado fruit is mainly sold fresh on the market, which however trades also a relevant quantity of second-grade fruits with relatively high oil content. The avocado oil contains all the beneficial attributes of the fruit, which makes it a very valuable product. It contains high amounts of the anti-cholesterol agent beta-sitosterol, a wide variety of vitamins and antioxidants, and other plant chemicals, which impart beneficial functional properties on humans. However, there is no widespread commercial method for oil recovery from avocado pulp. Traditionally, this oil is extracted from dried fruits by means of organic solvents, but a mechanical method is also used in general in locations where drying systems and/or solvent extraction units cannot be installed. These traditional processes yield a grade of oil that needs subsequent refining and is mainly used in the cosmetic industry. The particularity of the present work in Brazil appears in the new extraction methodology used, without usage of (enzyme, supercritical CO_2, acid hydrolysis, alkaline, solvent, heating, or add water); it relies on the separation of oil using centrifugal and mixing alternatively. The extracted oil is of high quality; a nice and attractive clear yellow or green color is Extra-Virgin Avocado Oil (EVAO) as it does not need to be refined. The oil is 100% natural, as well as the solids and the liquid; it keeps original nutritive ingredients, resulting into oil with characteristics (fatty acids, color) similar to those of Virgin olive oils. This method is simple, economic, fast and environment friendly.

Keyword: Avocado, cold- centrifuge, extra virgin avocado oil (EVAO), Brazil.

6.1. INTRODUCTION

Avocado (*Persea americana Mill*) is an oil-rich highly nutritious fruit. The pulp contains up to 30% of the oil. The avocado oil contains all the beneficial attributes of the fruit, which makes it a very valuable product. It contains high amounts of the anti-cholesterol agent, beta-sitosterol, a wide variety of vitamins and antioxidants, and other fruit chemicals, which impart beneficial functional properties on humans. Commercially avocado oil is extracted from the flesh of the fruit, similar to olive oil extraction, with the additional of two steps which includes the removal of skin and seed. After this step, the flesh is ground to a paste and then malaxed (mixing) for 40-60 minutes at 45-50°C. This is a higher malaxing temperature than the one used with olive oil extraction, but it is still considered to be cold-pressed extraction for avocado oil. Traditionally this oil is extracted from dried fruits by means of organic solvents. These traditional processes yield a grade of oil that needs subsequent refining and is mainly used in the cosmetic industry. The first attempt to develop a method to produce cold-pressed oil intended to obtain high-quality edible oil was made back in the late 1990's by a New Zealand company in collaboration with Alfa Laval (Eyres et al., 2001).

6.2. MATERIALS AND METHODS

Extraction of avocado oil by Cold- centrifuge

6.2.1. MATERIALS

This investigation was carried out at Tsuge Farm,in Rio Paranaíba, in Minas Gerais State (MG), Brazil. The located at Lat. 19° 25′ 33″S ; Long. 46° 15′ 37″ W. Altitude (11080m over sea level). By Köppen's classification the climate of the region is Aw type in transition to Cwb, with average annual minimum temperature of 11.0 °C. The mean annual temperature (MAT) averaged 21.1°C, mean annual maximum temperature of 22.3 °C and total annual precipitation of 2713.65 mm. (PIRES; YAMANISH, 2014).Mature avocado trees on ten to twelve years old of five cultivars; Hass, Breda, Margarida, Quintal, Fortuna. The trees were spaced 8 m X 6 m; not irrigated (in rainfed conditions) and received routine horticultural care (standard pest, disease &fertilizer programs). Avocados are harvested by hand from the trees. The mean yield (17.5/ 30/ 35/ 30/ 30) tone of fruit per hectare for Hass, Breda, Margarida, Quintal and Fortuna cultivars respectively.

Five avocado (*Persea americana Mill.*) cultivars: *Hass*, *Breda*, *Margarida*, *Quintal*, and*Fortuna* were randomly selected from the avocado orchard (Tusge) Minas Gerais, Rio Paranaíbain Brazil, in mid-August 2015. Fruits were mature and healthy, free of disease. Samples were saved in the cooling room temperature 10ºC humidity 85until maturity "can remove the shell by hand." avocado fruits as shown in table 10 (*Hass* 165- 315g; *Breda* 670-800g;*Quintal*965-1180g; *Margarida* 950-1215g; *Fortuna*890-1280g).
Mixer for home use (blender) made in Brazil;
Centrifugal mark Jouan BR4 made in Japan;
GC/MS Shimadzu-QP2010 Plus made in Japan;
Balance made in Germany;
Test Tubes; Plastic pipette; Knife;
Soxhlet apparatus mark Carvalhaes made in Brazil;
Rotary evaporator model Heidolph made in Germany.

6.2.2. METHODS

6.2.2.1. Dry matter contents, weight of fruit. Seed, peel, pulp, moisture percentage and oil content were determined on the samples of fruit, prepared from six fruits of each cultivar. Each fruit was cut into quarters, each quarter was cut into quarters, and the seed coat and peel removed 100%. Half the amount of pulp is placed in a blender to extract oil by cold-centrifugal. And the other half was placed in oven to extract oil by Soxhlet. Dry matter was determined by drying samples at 40 ^{o}C (5 - 6 days) to a constant mass.

6.2.2.2. Oil content: Oil content was determined by Soxhlet extraction, using Hexane as described by IUPAC (1979) method 1.122. The extraction solvent was removed by using rotary evaporator at 40 ^{o}C to recover the pulp oil. The oil was placed on a water bath at 50 ^{o}C for 2hours to ensure complete removal of residual solvent after which it was stored in a glass bottle, and the analysis was carried out on the freshly extracted pulp oils. An average of three replicates (triplicate) for each analysis were carried on. The oil content was calculated, then the percentage of oil yield.

6.2.2.3. Determination of fatty acid analysis**:**Fatty acid composition of avocado oil which extracted by centrifugal was determined as Fatty Acid Methyl Esters (FAMEs) according to the method described by IOC (2001) and were determined using gas chromatograph: Shimadzu GCMS-QP2010 equipped with a flame ionization detector (FID).

6.2.2.4. Statistical analysis**:** All analyses for parameters were carried out in triplicate and the results were expressed as the mean value± standard deviation for fatty acids by using SAS to evaluate the significance of differences between values at the level of$P < 0.05$.

6.3. RESULTS AND DISCUSSION

Design Process of cold-centrifuge:

The steps are as shown in table 9, and figure 4:

Fruit washing, destoning, deskinning and mash preparation. Whole fruits are washed to remove dust from the surface of the fruits and removed seeds and skin.

3.1.1. Crushing the pulp by using mixer for home use.

3.1.2. After crushing the pulp, the avocado mash is pumped into the centrifugal to separate and extract the oil. As seen in table (1) the optimal centrifuge time is 2-5 minutes two minutes for Hass and Breda cultivars, five minutes for Margarida, Quintal, and Fortuna cultivars. The temperature is 24°C to reach the best organoleptic characteristics (odor, taste and color) of Extra Virgin Avocado Oils (EVAO). The rotation speed is continuously at 10,000 rpm.

3.1.3. Remove the oil.

3.1.4. Remove the liquid (if any).

3.1.5. Mix the mash paste (solids).

3.1.6. Re-use the centrifugal again to get more yield of oil.

3.1.7. Remove the oil and mix the mash (paste) again.

Repeat the following steps 10 times, alternatively, in sequence: centrifuge / oil removal / liquid removal / mixing the paste to separate more oil.

Hass cultivar, the percentage of dry matter as shown in table 10and it was (34%). We got 2-phase of separation - oil and solids. However, with the cultivar *Breda*, the percentage of dry matter was (30%). At first centrifuge, we got three phases: oil on the top, solids, and liquid. It was better to remove the oil from the top, after that the liquid was remove and the remaining solids were centrifuged again until all the oil was removed. While cultivars *Margarida*, *Quintal*, and *Fortuna*, had the percentage of dry matter of 28%. At first centrifuge, we got only two phases: liquid and solids. We needed to remove the liquid three times, after that the solids were mixedand centrifuges, then three phases were obtained: oil, liquid, and solids. It was better to remove the liquid when separate, and remove the oil alternately.

Table (9) steps and design process of cold-centrifugal for avocado oil extraction for cultivars (Hass, Breda, Margarida, Fortuna, and Quintal).

	Avocado fruit *Hass*	Avocado fruit *Breda*	Avocado fruit *Margarida, Fortuna,* and *Quintal*
1	Water washing	Water washing	Water washing
2	De-stoning and peeling 100%	De-stoning and peeling 100%	De-stoning and peeling 100%
3	Crushing/ malaxing	Crushing/ malaxing	Crushing/ malaxing
4	Centrifugation 24ºC 2minutes/10,000 rpm	Centrifugation 24ºC 2minutes/10,000 rpm	Centrifugation 24ºC 5minutes/10,000 rpm
5	Solids → Oil	liquid ← solids → Oil	solids → liquid
6	Mixing	mixing	mixing
7	Centrifugation	Centrifugation	Centrifugation
8	Solids → Oil	liquid ← solids → Oil	solids → liquid
9	Mixing	mixing	mixing
10	Centrifugation	Centrifugation	Centrifugation
11	Solids → Oil	liquid ← solids → Oil	Oil ← solids → liquid
12	Mixing	mixing	mixing
13	Centrifugation	Centrifugation	Centrifugation
14	Solids → Oil	liquid ← solids → Oil	Oil ← solid → liquid
	Natural 100%		

Table (9) shows designing steps of Avocado Oil extracted using Cold-Centrifuge method for the cultivars *Hass*, *Breda*, *Margarida*, *Fortuna*, and *Quintal*. As we can see oil separation steps varied according to the cultivars type. With the Hass cultivar only oil was centrifuged out of the solid mix, However liquid, solid and oil were centrifuged out of the Breda cultivar. With the last three cultivars (*Margarida, Fortuna, and Quintal*), oil was separated during the last two steps only. In general, all three products: Liquid, solid and oil were naturally produced without any chemical or biochemical modification.

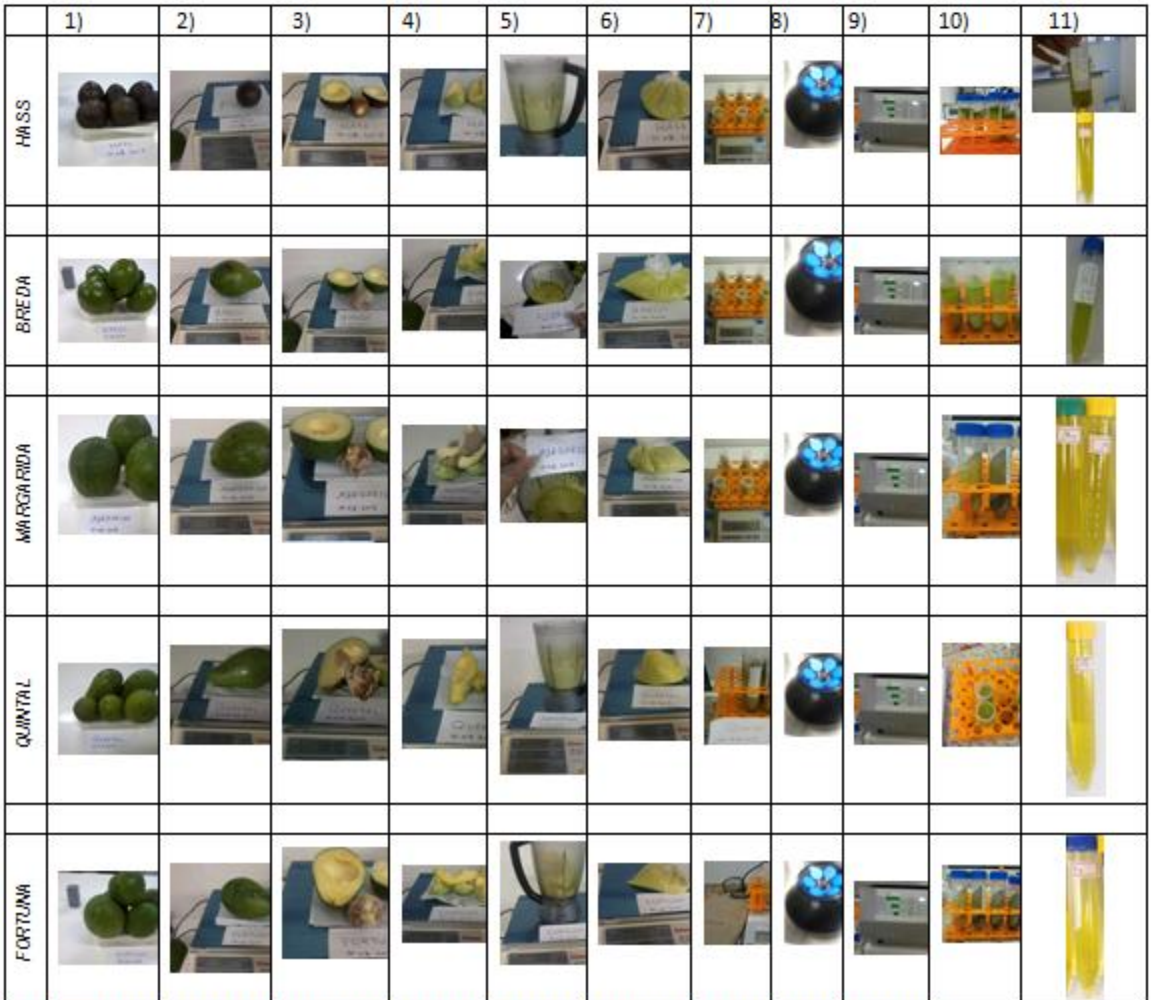

Figure (1) steps and design process of cold-centrifugal for avocado oil extraction for cultivars (Hass, Breda, Margarida, Fortuna, and Quintal).

Table 10 reports the characteristics of different fruit varieties harvested from the Minas Gerais, Rio Paranaíba farm (Tsuge) in Brazil, in mid-August 2015.It clearly shows an example of their variability particularly in terms of size, oil content, dry matter, seed, pulp, peel, moisture percentage and some physical and chemical characteristic. In general, the fruit is roughly pear-shaped in both Hass and Margarida cultivars, while smoothly pear-shaped in Breda, Fortuna and Quintal cultivars, more or less elongated. Its weight may range from 216.66 g in Hass cultivar the lowest weight while it is the highest level of oil content (25%) to 1091.0 g in Quintal cultivar. The relative amount of pulp varies according to the cultivar from 67.96% in Hass to 85.3% in Fortuna cultivar. The oil content may also vary widely (13.15% in *Margarida* to 25% in *Hass*) by soxhlet extraction, and (7.9% in *Margarida* to 20% in *Hass*) by cold-centrifugal. The oil texture at room temperature (25°C) were liquid in Hass cultivar and solid in other cultivars by

Soxhlet extraction. The oil which is extracted by cold-centrifugal is liquid for all cultivars.

The cold-centrifugal yield is 60% in *Margarida* and Quintal to 80% in *Hass* cultivar (Extra Virgin Avocado Oil extracted/oil content extracted by soxhlet) depending on the fruit cultivar.

Fatty acid analysis: Table 10 showsfatty acid composition of avocado oil samples. All oils of five cultivars are found to have oleic acid as the most dominant fatty acid. The main fatty acids composed of samples were oleic (C18:1), palmitic (C16:0), linoleic (C18:2) and palmitoleic acids (C16:1). These main fatty acidcomposition agreed with previous studies (Jorge, 2014;Haiyan et al., 2007; Moreno et al., 2003; Yanty et al., 2011). Table 10shows that the fatty acid composition of avocado oil samples especially Hass and Margarida is very similar to that reported by Jorge,2014.

All oils had high amounts of total monounsaturated fatty acids, *Quintal* (74.74%), *Breda* (72.59%) *Fortuna* (67.86%), *Hass* (66.08%), and *Margarida* (60.36%). Meanwhile, the highest level of saturated fatty acids is presented by *Margarida* (23.6%) followed by *Hass* (22.06%); *Fortuna* (20.15%); *Breda* (18.06%) and *Quintal* (16.46%). Fatty acid composition of avocado oil depends upon the cultivar, stage of ripening and the geographical growth location and different sample processing (Ahmed and Barmore, 1980; Bora *et al*., 2001; Moreno *et al*., 2003). In comparison, the fatty acid composition of olive oil is 55-83% oleic (C18:1); 7.5-20.00% palmitic (C16:0); 2.5-21.0% linoleic (C18:2) and 0.3-3.5% and palmitoleic acids (C16:1) IOC,2015. So we may name avocado as the " Brazilian Olive" in English language and as "Azeitona Brazileira" in either Arabic or Portuguese languages.

Table 10. Fruit characteristics of different varieties of avocado (*Hass, Breda, Fortuna, Quintal, and Margarida*), Fruit weight, pulp, seed, peel, oil and moisture percentage in fruits and Some parameters.					
cultivar	*Hass*	*Breda*	*Fortuna*	*Quintal*	*Margarida*
Fruit Weight (g)	216.66±85.49a	721.67±68.98b	1075.0±195.77c	1091±110.72c	1078.33±132.70c
Pulp (%)	67.96±7.37c	71.2±6.61b	85.3±1.32a	83.33±0.75a	84.66±2.25a
Peel (%)	12.2±0.7d	7.73±0.8c	6.03±0.21a	5.33±0.47b	6.46±0.42a
Seed (%)	18.26±2.82d	21.06±2.33c	8.66±2.21a	11.33±2.85b	8.63±4.8a
Dry matter (%)	34.11±2.82e	30.34±2.33d	22.25±2.21c	28.49±2.85b	20.29±4.8a
Oil content (%)v/w Cold-centrifugal	20±0.5a	15.5±0.6b	9±0.3c	9.7±0.6d	7.9±0.3e
Texture at room temperature (25°C)	Liquid	Liquid	Liquid	Liquid	Liquid
color	Light greenish-yellow	Light greenish-yellow	Light yellow	Light yellow	Light yellow
Oil content (%)v/w soxhlet	25.00±1.2	22.11±0.8	13.26±0.6	16.13±0.8	13.15±0.5
Texture at room temperature (25°C)	Liquid	Solid	Solid	Solid	Solid
color	Light brown	Light green	Light green	Light green	Light green
Efficiency (Cold-centrifugal/ soxhlet) %	80	67.87	67.87	60.14	60
Fatty acids(%) média					
Palmitic acid (16:0)	21.37±0.01a	18.06±0.03b	19.57±0.03c	16.46±0.02d	23.26±e
Palmitoleic acid (16:1)	12.86±0.00d	6.56±0.01c	2.36±0.01b	4.36±0.01a	4.04±a
Stearic acid (18:0)	0.69±0.02a	-	0.58±0.00b	-	0.34±c
Oleic acid (18:1)	53.22±0.04a	66.03±0.04b	65.50±0.05c	70.44±0.05d	56.32±e
Linoleic acid (18:2)	11.86±0.02a	9.35±0.02b	11.99±0.03a	8.30±0.01b	15.13±c
Linolenic acid (18:3)				0.44±0.00	0.91±01
Saturated (SFAs)	22.06	18.06	20.15	16.46	23.60
Monounsaturated (MUFAs)	66.08	72.59	67.86	74.80	60.36
polyunsaturated (PUFAs)	11.86	9.35	11.99	8.74	16.04

Mean ± standard deviation followed by the same letters in the lines do not differ by Tukey test ($p<0.05$).

6.4. CONCLUSION & RECOMMENDATIONS

- This paper presents the progress of a research work focused on developing a new Avocado Oil-Extraction process that could be applied anywhere.
- The final products of the extraction process are oil, liquid, and paste that are 100% natural. Only (mechanical separation of the oil, fruit water, and paste phases at 24°C to avoid any thermal degradation) is used; it keeps original nutritive ingredients. The oil is of high quality; a nice and attractive clear yellow or green color; is the Extra-Virgin Avocado Oil (EVAO) as it does not need to be refined; its characteristics (fatty acids, color) are similar to those of Virgin olive oils, however, the yield is (60- 80 %).
- This method is simple, economic, fast and environment friendly.
- The method could be adapted on an industrial scale, resulting in the richest quality of oil as compared with the refined oil.
- The extraction of oil by cold-centrifuge process may retained all the natural flavor and nutrition, in addition to being rich and healthy.

6.5. REFERENCES

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7. EXPERIMENT- III

INFLUENCE OF DIFFERENT CULTIVARS AND HARVESTING TIME OF AVOCADO FRUIT ON OIL CONTENT, DRY MATTER, AND FATTY ACID COMPOSITION

Abstract

Oil content, dry matter, fatty acid composition and fruit weight of three Brazilian avocado (*Persea Americana*, *Mill*) cultivars: *Hass*, *Quintal* and *Fortuna* were examined during three harvesting time. Fruits were harvested in mid-June, July and August 2015. Avocado fruits were harvested according to Tsuge Farm picking standard in Rio Paranaíbacity, Minas Gerais State, Brazil. Samples were kept under ambient conditions to ripen. Oil content (fresh weight basis) of *Hass, Quintal* and *Fortuna* increased significantly ($P < 0.05$), from 22.4% to 25.6%, from 16.3% to 19.8% and from 9.8% to 13.3%, respectively, according to the length of time that the fruits remained on the tree. Significant differences in oil content among cultivars were observed at the same harvesting time. The highest percentage of oil was observed in *Hass,* while the lowest percentage was noticed in *Fortuna*. Moreover, significant differences were observed in dry matter among the three cultivars. *Hass* had significantly higher dry matter (32.9% to 34.1%) than the other cultivars, while *Fortuna* (20.2% to 23.2%) had the lowest. The average fruit weight was significantly influenced by the cultivar type. The highest average fruit weight observed for *Quintal* (720g to 1091.7g) and *Fortuna* (816.7g to 1075g) while the lowest was for *Hass* (218.3g to 338.3g). For all cultivars, oleic (54.01/65.9/64.22%), palmitic (21.18/ 17.96/19.09), linoleic (11.98/11.45/13.96%) and Palmitoleic (12.83/ 4.69/1.77%) acids were the major fatty acids found in *Hass*, *Quintal* and *Fortuna*, respectively. The saturated fatty acid of*Hass*, *Quintal* and *Fortuna* increased significantly, from 21.18% to 22.05%, from 17.96% to 21.84% and from 19.09% to 21.84% during harvesting time, respectively. There was very little variation in the content of monounsaturated fatty acids (MUFAs) of the pulp oils between the cultivars, while the polyunsaturated fatty acids (PUFAs) decreased in general.

***Key words*:** *Hass*, *Quintal*, *Fortuna,* Brazil, MUFAs, PUFAs, PUFAs.

7.1. INTRODUCTION

The avocado (Persea americana Mill.) is a polymorphic tree species that originated in a broad geographical area from the Eastern and central highlands of Mexico through Guatemala to the Pacific (Storey et al., 1986; Bost et al., 2013; Athar and Nasir, 2005). It is belonging to the Lauraceae family and Persea genus (Maranca, 1980; Koller, 1992). It is one of the most productive plants per unit of cultivated area. A large number of varieties of avocado are found in different regions of Brazil, whose fruits have varied chemical composition, especially in terms of levels of lipids in the pulp. Fruits with high levels of lipids in the pulp can be important raw material for oil extraction (Tango; Turatti, 1992).

Currently, avocado is a fruit that has been cultivated in many parts of the world, especially tropical countries. Avocado fruits are existed in different shape, size, color, depending on their variety. Avocado fruit can be consumed directly as a high energy food source because of its content of lipids that are significantly higher than those in other fruits. Besides, avocado fruit is also a good source of oil (Quinones-Islas et al., 2013). Avocado oil has been reported to lower cholesterol level (Moreno et al., 2003), maintain skin elasticity (Athar and Nasir, 2005) and reduce the coronary heart risk (Berasategi et al., 2012). Avocado oil is also widely used in the food industry, cosmetics and health products because of its unique characteristics and functions (Swisher, 1988), especially because of its high content of unsaturated fatty acid. Unsaturated fatty acids include monounsaturated fatty acids (MUFAs), approximately 71%, which is dominated by oleic acid and polyunsaturated fatty acids (PUFAs) which is amounted to 13% of the total fat (Lu et al., 2009). Avocado oil content is highly correlated to fruit dry matter content (Lee et al., 1983; Tango et al., 2004). As fruit mature, the percentage of dry matter increases, as does the oil content; however, there is cultivar-to-cultivar variation in this relationship. Percentage of dry matter content has become the predominant maturity index for avocado harvesting, but it must be cultivar-specific (Bower and Cutting, 1988; Hofman et al., 2002; Lee et al., 1983). California, Australia, and Brazil also use percentage of dry matter content as an indirect measure to determine oil content, and hence maturity, for different cultivars (Lee and Coggins, 1982; Woolf et al., 2004).

Oil content and dry matter content are keys component of avocado flavor and texture. The Hawaii Department of Agriculture for example specifies a minimum of 12% oil content for "Hawaii Fancy" and "Hawaii No. l" grades. Avocado cultivars vary widely in fruit oil content, from 5% to > 30% (Woolf et al., 2004).

The avocado fruit is classified into three races: West Indian, Mexican, and Guatemalan (Morton, 1987). The Fortuna cultivar is Guatemalan x West Indian hybrids, which plays an important role in the Florida avocado industry. The

Fortuna cultivar is a large fruit with dark green skin and yellow pulp, and it contains about 16% oil (Brasil, 1971; Medina, 1980). In Brazil, the fruit is classified into two groups (Brasil, 1971): the fruits in the first group, which includes the Collinson and Barker cultivars, are suitable for long-distance transport and are commercialized as whole fruit, while the fruits in the second group, which includes the Fortuna cultivar, are delicate and have buttery consistency and soft pulp texture so that they are more suitable for oil extraction. The avocado oil, which is similar in composition to olive oil, is highly digestible and consists mainly of unsaturated fatty acids, predominantly oleic acid (Gómez-López, 1998, 1999), which contributes to the consistency and the special taste of the fruit (Sinyinda & Gramshaw, 1998). The avocado oil content is used as a parameter to evaluate the maturation stage of the fruit for harvest purposes (Donadio, 1995). For this reason, the lipid fraction of the avocado fruit has been studied by several authors, focusing on the composition of its fatty acids (Bora et al., 2001; Frega et al., 1990; Freitas et al., 1993; Martinez Nieto et al., 1988; Ratovohery et al., 1988; Soares et al., 1991; Southwell et al., 1990).

Description of the studied cultivars: Knowing the variety of avocado that one intends to cultivate is very important, since each market has its particular preferences. Ramos and Sampaio (2008) cite that the national market prefers large fruits with little amount of oil. On the other hand, in the European market; small fruit with a high oil content has greater acceptance. In addition, it is great importance to keep in mind the different characteristics among such varieties to make a good planning from the implantation of the crop to the flow of production to the market for which it is intended. Some important characteristics are described below:

Hass: It is a Guatemalense- mexicanohybrid that belongs to floral group A. The fruit is oval-pyriform, and has a thick and rough skin that confers a good resistance to transport. It weighs from 180 to 300 grams, and the pulp is of good quality and without fibers (Donadio, 1995).

Quintal: It is a hybrid Antilhano- guatemalense, of the floral group B. It has pyriform fruit, it weighs 600 to 800 grams (Donadio, 1987). The harvest period varies from April to June, has high yield of pulp and medium yield of oil.

Fortuna: It is a hybrid of the races Antilhana x Guatemalense that belongs to the floral group A. Its fruit is big, weighing 750 grams in average; Egg-yellow pulp, somewhat sweet. The production season varies from May to August. It is one of the most planted cultivars for domestic consumption (Teixeira, 1991).

Ripening stage is used mainly as a guideline to establish the avocado harvest time.

to date, there is no investigation on effect of cultivar and harvesting time of Brazilian avocado fruit on oil content; dry matter of pulp and fatty acid composition of that extracted oil. The objectives of current research were to determine effects of different cultivars and harvesting time of Brazilian avocado fruit (*Persea americana Mill*.) on oil content, dry matter, and fatty acid composition of that extracted oil which help in determining the proper harvesting time and to have information if the selected cultivars are proper to extract oil.

7.2. MATERIALS AND METHODS

7.2.1. MATERIALS

This investigation was carried out at Tsuge Farm, in Rio Paranaíba, in Minas Gerais State (MG), Brazil. The located at Lat. 19° 25′ 33″S ; Long. 46° 15′ 37″ W. Altitude (11080m over sea level). By Köppen's classification the climate of the region is Aw type in transition to Cwb, with average annual minimum temperature of 11.0 ° C. The mean annual temperature (MAT) averaged 21.1°C, mean annual maximum temperature of 22.3 ° C and total annual precipitation of 2713.65 mm. (PIRES; YAMANISH, 2014). Mature avocado trees on ten to twelve years old of three cultivars; Hass, Quintal and Fortuna. The trees were spaced 8 m X 6 m; not irrigated and received routine horticultural care (standard pest, disease &fertilizer programs). Avocados are harvested by hand from the trees. The mean yield (17.5/ 30/ 30) tone of fruit per hectare for Hass, Quintal and Fortuna cultivars respectively.

Three Brazilian avocado cultivars, Hass, Quintal, and Fortuna (Persea americana Mill.), were selected for this experiment. Mature avocado fruits without infection or physical damages were picked up in the second week of June, July, and August, 2015, according to Tsuge Farm picking standard in Rio Paranaíbacity, Minas Gerais State, Brazil. Sufficient fruits were handpicked from the trees; fruits from each cultivars were placed in single layer trays and transported to the laboratory within few hours, and no maturation inhibitor or accelerator was used. Fruits free from any apparent skin damage were selected for analysis.

7.2.2. METHODS

7.2.2.1.Dry matter contents and fruit characteristics:

The pulp was manually removed and homogenized using a domestic blender. Dry matter of pulp was determined by drying samples to a constant mass. The peel and seed were separated manually. The malaxed pulp was packaged in polyethylene bags and stored in a refrigerator (4±1°C) until the oil was extract by

Soxhlet which was conducted within one day. Fresh weight of fruit , pulp, seed and peel percentage for selected fruits were determined on the samples, which were prepared from six fruits of each cultivar, during three harvesting time.

7.2.2.2. Oil content:

Oil content was determined by Soxhlet extraction, using Hexane as described by IUPAC (1979) method 1.122. Dried ground samples (10 g) were placed into the extractor with 250 mL of Hexane and were extracted for 6 hours. After extraction, the samples of mixture Hexane with oil were transferred to a rotary evaporator to separate the hexane from oil at 40 ºC, and percentage of oil content was calculated. Oil content was expressed as both % f.w (fresh weight) and % d.w (dry weight) in pulp. The solvents were of pure grade (purity >97.7%) and were obtained from Merck and Brazil.

7.2.2.3. Fatty acid composition of the extracted oil:

Fatty acids were transformed into methyl esters (FAME) according to the method described by IOC (2001) and were determined using Gas Chromatograph: Shimadzu GCMS-QP2010 equipped with a flame ionization detector (FID).

7.2.2.4. Statistical analysis

The results of the three samples analyzed in duplicate (n=6) were processed for the determination of mean and standard deviation values using the SAS software (SAS Institute, Cary, NC) Version 9.1.3. Significant differences between the mean values of different characteristics were determined by the Tukey's test for multiple comparisons at the probability of 5% ($p < 0.05$).

7.3. RESULTS AND DISCUSSION

The data of the physical composition of the avocado fruits of the three cultivars Hass, Quintal and Fortuna during three harvesting time (June, July and August) are presented in Table 11. The mean fruit weight during three harvesting time; The weight of fruits of the Fortuna (927.0g) and Quintal (848g) cultivars were significantly heavier than the fruits of the Hass (272g) cultivar during all harvesting time. Medina (1980) reported an average weight of 718g for the Fortuna cultivar in fruits grown in the state of São Paulo, Brazil. While, Silva (2011) reported an average weight of (900/ 1100/ 200g) for fruits of the Fortuna, Quintal and Hass cultivars, respectively, which were collected from the municipality of Carmo da Cachoeira in the state of Minas Gerais, Brazil. However, Galvão et al (2014) reported an average weight of (418.33g) for fruits of the Fortuna cultivar which was obtained from the Experimental Station of Itambé (IPA - region in the Pernambuco, Brazil). The maximum pulp yield was also obtained for the Fortuna and Quintal cultivar fruits (81.4/ 81.0 %), respectively. While the minimum pulp yield was (67.6%) in Hass cultivar. Moreover, Silva (2011) reported an average pulp yield of (78.6/ 81.6/ 59.9%) in Fortuna, Quintal and Hass cultivar fruits, respectively. While Galvão et al (2014) reported (75.5%) for the Fortuna cultivar. The highest seed content was in Hass 19.7%, while it was 13% in Quintal and 12.0% in Fortuna cultivars. However, Galvão et al (2014) reported higher (15.14%) seed content in fruits of the Fortuna. While Silva (2011) reported an average seed content of (11.3/ 10.6/ 25.7%) for fruits of the Fortuna, Quintal and Hass cultivars, respectively. As well as, peel content; the highest percentage was in Hass 12.6%. while, 6.0% in Quintal and 6.6% in Fortuna. Galvão et al (2014) reported higher (9.16%) in fruits of the Fortuna. While Silva (2011) reported an average peel content of (8.8/ 5.6/ 16.7%) for fruits of the Fortuna, Quintal and Hass cultivars, respectively.

Table 11 shows that the dry matter and the oil content of all cultivars (Hass, Quintal and Fortuna) of pulp fruits increased from June to August. The dry matter content of pulp increased in rate of (3.64/6.11/14.85%) from June to August in Hass, Quintal and Fortuna cultivars, respectively. Moreover, the amount of oil similarly increased in the Hass, Quintal and Fortuna fruits from June to August at rates of (14.29/ 21.47/ 35.71%), respectively. Gaydou et al. (1987) reported that the moisture content of avocado mesocarp decreased steadily with increasing lipid content during the 12–39 weeks after flowering. While Ozdemir and Topuz (2004) reported increase in the dry matter contents of Fuerte and Hass cultivars at rate of (25.1/ 43.6%) from November to January, respectively. The oil content increased from November to January at the rates of 23.74% and 77.5% in Fuerte and Hass, respectively. Dry matter and oil content shows variations due to cultivars and harvesting time, which produce moisture losses.

Table 11. Values (mean ± standard deviation) of fruit parameters from *'Hass'*, *'Quintal'* and *'Fortuna'* avocado cultivars for three different harvesting time.						
parameter	**Cultivar**	**Harvesting Time**			**Mean**	**Range**
		June	**July**	**August**		
Weight (g)	***Hass***	260.0±20.0b/C	338.3±16.1a/B	218.3±83.9b/B	272A±45ab/B	218.3-338.3
	Quintal	720.0±30.5b/B	733.0±155.4b/A	1091.7±110.7a/A	848B±115ab/A	720-1091.7
	Fortuna	890.0±40.0a/A	816.7±144.7a/A	1075.0±195.8a/A	927B±145a/A	816.7-1075.0
Pulp (%)	***Hass***	66.4±2.5a/B	68.9±1.9a/B	67.5±5.5a/C	67.6A±3.9a/B	66.4-68.9
	Quintal	78.4±1.2c/A	81.4±1.2b/A	83.3±0.7a/B	81.0B±1.9b/A	78.4-83.3
	Fortuna	78.0±2.3c/A	81.0±2.2b/A	85.2±1.2a/A	81.4B±1.8b/A	78.0-85.2
Seed (%)	***Hass***	20.5±0.9a/A	18.8±1.6a/A	19.9±5.9a/A	19.7A±2.6a/A	18.8-20.5
	Quintal	16.2±2.7a/B	11.5±2.3b/B	11.3±0.9b/B	13.0±1.8b/B	11.3-16.2
	Fortuna	15.5±1.9a/B	11.7±1.2b/B	8.7±1.1c/C	12.0B±1.2b/B	8.7-15.5
Peel (%)	***Hass***	13.1±0.8a/A	12.3±2.1a/A	12.6±1.4a/A	12.7±1.2a/A	12.3-13.1
	Quintal	5.4±0.6/a/B	7.1±1.9a/B	5.4±0.4a/B	6±0.8a/B	5.4-7.1
	Fortuna	6.5±0.9a/B	7.3±1.3a/B	6.1±0.2a/B	6.6±0.7a/B	6.1-7.3
Pulp dry matter (%)	***Hass***	32.9±0.41b/A	33.3±0.62b/A	34.1±0.81a/A	33.43±0.72b/A	32.9-34.1
	Quintal	26.2±0.23a/B	27.0±0.32a/B	27.8±0.57a/B	27.00±0.53a/B	26.2-27.8
	Fortuna	20.2±0.15c/C	21.2±0.25b/C	23.2±0.31a/C	21.53±0.22b/c	20.2-23.2
Pulp oil content (FW) (%)	***Hass***	22.4±0.41c/A	25±0.11b/A	25.6±0.13a/A	24.33±0.36b/A	22.4-25.6
	Quintal	16.3±0.24b/B	19.6±0.27a/B	19.8±0.35a/B	18.57±0.31a/B	16.3-19.8
	Fortuna	9.8±0.19c/C	10.5±0.20b/C	13.3±0.15a/C	11.2±0.17b/C	9.8-13.3
Pulp oil content (DW) (%)	***Hass***	68.1±1.65c/A	75.1±0.82a/A	75.1±0.93a/A	72.77±1.25b/A	68.1-75.1
	Quintal	62.2±1.54c/B	72.6±1.51a/B	71.2±1.31a/B	68.67±1.11b/B	62.2-71.2
	Fortuna	48.5±0.82c/C	49.5±1.33c/C	57.3±0.9a/C	51.77±1.09b/C	48.5-57.3
Mean values followed by different uppercase and lowercase indicate significant statistical differences ($p<0.05$) between and within cultivars during harvesting time, respectively, according to the Tukey test.						

Fatty acid analysis: Table (12) showed the fatty acids composition of avocado oil samples of three cultivars during three harvesting time. All oils of three cultivars during all harvesting time are found to have oleic acid as the most dominant fatty acid (52.69-54.01 / 63.97-65.90 / 62.88-64.22%), followed by palmitic (21.18-21.51 / 17.96-21.84 / 19.09-21.84%), linolenic (10.19-11.98 / 9.64-11.45 / 12.02-13.96%), and palmitoleic (12.83-14.8 / 4.13-4.69 / 1.77-2.44%) acids which were found to be the major fatty acids in avocado oil of *Hass, Quintal* and *Fortuna* cultivars, respectively. The highest value of Palmitoleic acid during three harvesting time was found in *Hass* (12.83 to 14.8%), followed by *Quintal* (4.13-4.69%). while, the lowest percentage (1.77 to 2.44%) was found in *Fortuna* cultivar.

Fatty acid composition of avocado oil depends upon the cultivar, stage of ripening, the geographical growth location and different sample processing (Ahmed and Barmore, 1980; Bora et al., 2001; Moreno et al., 2003). These main fatty acids composition agreed with previous studies (Jorge, 2014;Haiyan et al., 2007; Moreno et al., 2003; Yanty et al., 2011). Stearic acid was also determined to be in small amounts only in August in *Hass* cultivar and in July in *Quintal* cultivar and was not presented in other harvesting time. while it was not presented in all harvesting time in *Fortuna* cultivar, and Linolenic acid was also determined to be in small amounts in July and August in *Hass* cultivar and only in June in *Fortuna* cultivar. However, it was not presented in all harvesting time in the *Quintal* cultivar. Based on the linoleic and linolenic acid contents in the oils of the three avocado cultivars, it can be concluded that these cultivars are rich sources of these ω-3 and ω-6 fatty acids, which could contribute towards the daily needs of an adult (0.8 to 4.0g), (World Health Organization & Food and Agriculture Organization, 2003). Other researchers (Brasil, 1971; Bora et al., 2001; Frega et al., 1990; Martinez Nieto et al., 1988; Medina, 1980; Moreno et al., 2003; Ratovohery et al., 1988; Salgado et al., 2008; Tango et al., 1972, 2004; Villa-Rodríguez et al., 2011; Dreher & Davenport, 2013) have also reported oleic acid as the major fatty acid, followed by palmitic, linolenic, and palmitoleic acids in the pulp of Collinson, Barker, Fortuna, Lula, Bacon, Fuerte, Zutano, Hass, and Margarida cultivars of avocado fruit.

In general, oleic acid was the fatty acid which decreased from June to August in both Hass and Quintal cultivars, with percentages ranging from 54.01% to 52.69% in the *Hass*, and from 65.9% to 63.97% in the *Quintal* cultivar. while, in Fortuna fluctuated during three harvesting time. But, Linoleic acid which decreased from June to August in all cultivars *Hass*, *Quintan* and *Fortuna* from 11.98% to 10.19%, from 11.455 to 9.64% and from 13.96% to 12.025, respectively. In contrast, Palmitic acid steadily increased from June to August in *Hass*, *Quintal*, *Fortuna* cultivars from 21.18% to 21.51%, from 17.96% to 21.84% and from 21.23% to 21.84%, respectively.

Table 12. Fatty acid composition (percent) of pulp oils obtained from three different cultivars of Brazilian avocado fruit during three harvesting time.

Fatty acids	Cultivars	Harvesting Time				
		June	July	August	mean	Range
C16:0 Palimitic acid	*Hass*	21.18±0.33 a/A	21.37±0.23a /A	21.51±0.12a /B	21.35±0.15 a/A	21.18-21.51
	Quintal	17.96±0.0.14c/C	18.21±0.13c /C	21.84±0.23a /A	19.34±0.15 b/C	17.96-21.84
	Fortuna	19.09±0.19 d/B	21.23±0.15b /B	21.84±0.25a /A	20.72±0.18 c/B	19.09-21.84
C16:1 Palmitoleic acid	*Hass*	12.83±0.07 c/A	12.86±0.09c /A	14.80±0.10a /A	13.5±0.81b /A	12.83-14.8
	Quintal	4.69±0.02a /B	4.13±0.03d/ B	4.55±0.06b/ B	4.46±0.02c /B	4.13-4.69
	Fortuna	1.77±0.02d /C	2.2±0.06b/C	2.44±0.05a/ C	2.14±0.02c /C	1.77-2.44
C18:0 Stearic acid	*Hass*	-	-	0.54±0.01a/ A	0.18±0.01b /A	0.00-0.54
	Quintal	-	1.07±0.01a/ A	-	0.36±0.01b /B	0.00-1.07
	Fortuna	-	-	-	-	-
C18:1 Oleic acid	*Hass*	54.01±0.45 a/C	53.22±0.33b /C	52.69±0.23c /C	53.31±0.35 b/C	52.69-54.01
	Quintal	65.9±0.26a /A	65.44±0.39b /A	63.97±0.46c /A	65.10±0.35 b/A	63.97-65.90
	Fortuna	64.22±0.51 a/B	62.88±0.82b /B	63.7±0.74a/ B	63.60±0.65 a/B	62.88-64.22
C18:2 Linoleic acid	*Hass*	11.98±0.04 a/B	11.86±0.02a /B	10.19±0.07c /B	11.34±0.08 b/B	10.19-11.98
	Quintal	11.45±0.03 a/C	11.15±0.04a /C	9.64±0.06c/ C	10.75±0.08 b/C	9.64-11.45
	Fortuna	13.96±0.06 a/A	13.69±0.09b /A	12.02±0.8d/ A	13.22±0.07 c/A	12.02-13.96
C18:3 Linolenic acid	*Hass*	-	0.69±0.02a/ A	0.27±0.01b/ A	0.32±0.01b /A	0.00-0.69
	Quintal	-	-	-	-	-
	Fortuna	0.96±0.01a /A	-	-	0.32±0.01b /A	0.00-0.96

ND- Not Identified
Each value in the table represents the mean ±standard deviation. Different uppercase and lowercase indicate significant statistical differences ($p<0.05$) between and within cultivars during harvesting time, respectively, according to the Tukey test.

Figure 5. Show the maximum value of oleic acid in the mean of three harvesting time was found in *Quintal* 65%, followed by *Fortuna* 63.6%. while, the

lowest percentage 53.31% was found in *Hass* cultivar. As known Oleic acid, like other omega-9s fatty acids, they all improve the Heart Health & Lower the bad Cholesterol (LDL). Oleic acid, can help in reducing the risk of heart disease by raising levels of high-density lipoprotein (HDL), “good cholesterol.” The oleic acid in avocado oil is also beneficial because it can lower low-density lipoprotein (LDL), “bad” cholesterol (Wood et al. (2003).

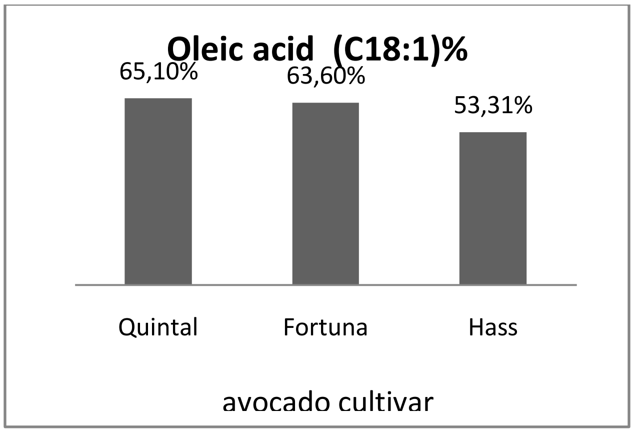

Figure 5. The percentage of Oleic acid of avocado oil (Hass, Quintal and Fortuna) at three harvesting time.

The total values of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated (PUFA), and Unsaturated fatty acids (UFA) in addition to the MUFA/SFA, PUFA/SFA, and UFA/SFA ratios during three harvesting time and for three cultivars are presented in Table 13. Avocado oil samples had high amounts of total unsaturated fatty acids during three harvesting time, i.e., *Hass* (77.95-78.81%), *Quintal* (78.16-82.04%) and *Fortuna* (78.16-80.91%), respectively. All the cultivars during three harvesting time were found to be healthy, as they had high MUFA: SFA ratio; (3.06-3.16/3.14-3.93/3.03-3.46) in *Hass*, *Quintal* and *Fortuna*, respectively. Moreover, and from table (5 and 6). In general it was noticed that there have been no clear cut variation (Ascending-Descending) in the fatty acid contents during storage periods. Also, there was no relation between molecular weight and degree of unsaturation, and the variations in these fatty acid contents with different storage periods, from different cultivars.

Wood et al. (2003) reported the importance of high ratio of PUFA/SFA to reduce cardiovascular diseases, and they recommended a minimum value of 0.4. Thus, higher values of this index in almost all oils extracted from the pulp of the avocado three cultivars, during three harvesting time show the high quality of the oils.

Table 13. Saturated (SFAs), Monounsaturated (MUFAs), Polyunsaturated (PUFAs), Unsaturated Fatty Acids (UFAs) profile of pulp oils obtained from three different cultivars of Brazilian avocado fruit during three harvesting time.

Cultivar	Fatty acids	Harvesting Time			Mean	Range
		June	July	August		
Hass	**SFAs**	21.18±0.16b/A	21.37±0.25b/A	22.05±0.18a/A	21.53±0.23b/A	21.18-22.05
	MUFAs	66.83±0.35b/B	66.08±0.29c/B	67.49±0.39a/B	66.80±0.26b/B	66.08-67.49
	PUFAs	11.98±0.13b/B	12.55±0.21a/B	10.46±0.15c/B	11.66±0.19b/B	10.46-12.55
	UFAs	78.81±0.71a/C	78.63±0.52a/B	77.95±0.39b/B	78.46±0.42a/C	77.95-78.81
	MUFAs/SFAs	3.16±0.03a/C	3.09±0.02b/B	3.06±0.01c/B	3.10±0.04b/C	3.06-3.16
	PUFAs/SFAs	0.57±0.01a/C	0.59±0.03a/B	0.47±0.02b/B	0.54±0.03a/B	0.47-0.59
	UFAs/SFAs	3.72±0.03a/C	3.68±0.05b/B	3.54±0.01c/A	3.65±0.04b/C	3.54-3.72
Quintal	**SFAs**	17.96±0.25c/C	19.28±0.18b/B	21.84±0.31a/A	19.69±0.27b/C	17.96-21.84
	MUFAs	70.59±0.34a/A	69.57±0.29b/A	68.52±0.51c/A	69.56±0.33b/A	68.52-70.59
	PUFAs	11.45±0.22a/C	11.15±0.18b/C	9.64±0.32d/C	10.75±0.25c/C	9.64-11.45
	UFAs	82.04±0.36a/A	80.72±0.52b/A	78.16±0.27c/A	80.31±0.39b/A	78.16-82.04
	MUFAs/SFAs	3.93±0.02a/A	3.61±0.01b/A	3.14±0.5d/A	3.56±0.07c/A	3.14-3.93
	PUFAs/SFAs	0.64±0.04a/B	0.58±0.01b/B	0.44±0.01c/B	0.55±0.03b/B	0.44-0.64
	UFAs/SFAs	4.57±0.03a/A	4.19±0.01b/A	3.58±0.02c/A	4.11±0.02b/A	3.58-4.57
Fortuna	**SFAs**	19.09±0.20d/B	21.23±0.18b/A	21.84±0.29a/A	20.72±0.23c/B	19.09-21.84
	MUFAs	65.99±0.81a/B	65.08±0.35b/C	66.14±0.37a/C	65.74±0.33a/C	65.08-66.14
	PUFAs	14.92±0.17a/A	13.69±0.21b/A	12.02±0.19c/A	13.54±0.25b/A	12.02-14.92
	UFAs	80.91±0.93a/B	78.77±0.66b/B	78.16±0.39c/A	79.28±0.72b/B	78.16-80.91
	MUFAs/SFAs	3.46±0.02a/B	3.07±0.01c/B	3.03±0.04c/B	3.18±0.01b/B	3.03-3.46
	PUFAs/SFAs	0.78±0.03a/A	0.64±0.02b/A	0.55±**0.01c/A**	0.66±0.01b/A	0.55-0.78
	UFAs/SFAs	4.24±0.03a/B	3.71±0.05b/B	3.58±0.03c/A	3.84±0.04d/B	3.58-4.24

SFAs: Saturated fatty acids (C16:0; C18:0)
MUFAs: Monounsaturated fatty acids (C16:1; C18:1)
PUFAs: Polyunsaturated fatty acids (C18:2; C18:3)
Each value in the table represents the mean ±standard deviation. Different uppercase and lowercase indicate significant statistical differences (p<0.05) between and within cultivars during harvesting time, respectively, according to the Tukey test.

7.4. CONCLUSION

The current research provide information about the physical composition of three cultivars of the Brazilian avocado fruits. As well as the dry matter and the oil content of pulp ,and fatty acid composition of that extracted oil, and how they are influenced by harvesting time. Oil content and dry matter differ according to cultivar and harvesting time. The oil content was significantly different ($p<0.05$) for all cultivars. The highest value for oil content was observed in Hass followed by Quintal. The highest percentage of pulp were noticed in Quintal and Fortuna. the highest fruit yield (tone per hectare) were found in Quintal and Fortuna. All selected cultivars are excellent to extract oil because of the high percentage of oil in the pulp, high percentage of pulp in the fruits and high yield of fruits per hectare. This study reveals that oleic acid was major fatty acid in all harvesting times for all cultivars. Oil content in pulp was lower in June and higher in august for all three cultivars. This concludes that the optimal harvesting time is by waiting until the pulp's oil content increases as described previously. The harvesting time should be achieved from the middle of July in order to obtain the maximum oil yield.

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8. EXPERIMENT. IV

EFFECTS OF CULTIVAR AND RIPENING STAGE OF BRAZILIAN AVOCADO FRUIT ON OIL CONTENT, DRY MATTER, AND FATTY ACID COMPOSITION

Abstract

The effects of different cultivars and ripening stages of Brazilian avocado fruits (Persea Americana, Mill) on oil content, dry matter, and fatty acid composition were evaluated. Three cultivars of avocado fruits including Hass, Breda and Margarida were harvested at four different ripening stages from June to September, 2015, according to Tsuge Farm picking standard in Rio Paranaíba city, Minas Gerais State, Brazil. Dry matter content in Hass, Breda and Margarida increased from 32.93% to 36.24%, from 23.81% to 31.37% and from 18 % to 23%, respectively, during ripening stages. Oil content increased significantly ($p<0.05$) from 22.38% to 26.04% in Hass, from 11.8% to 23.9% in Breda and from 8.04% to 14.37% in Margarida. In all ripening stages, of all cultivars, oleic (52.83 / 65.36 / 57.71%), palmitic (21.48 / 19.34/ 23.94%), linoleic (12.13 / 9.41 / 14.21%) and palmitoleic (13.2 / 5.58 / 3.5%) acids were the major fatty acids, in Hass, Breda and Margarida, respectively.

Keywords: Avocado, *Hass*, *Breda*, *Margarida*, fatty acid composition, ripening stage.

8.1. INTRODUCTION

The avocado tree is a fruit plant originated in the Americas, especially Mexico and Central America, belonging to the *Lauraceae* family and *Persea* genus (Maranca, 1980; Koller, 1992). Mexico is the country that leads the production of avocados in the world. In Brazil, The state of São Paulo is the largest domestic producer, accounting for more than 50% of production. The state of Minas Gerais is the second, followed by Paraná and Espírito Santo (Agrianual, 2016). It is one of the most productive plants per unit of cultivated area (Schaffer et al., 2013). A large number of varieties of avocado are found in different regions of Brazil, whose fruits have varied chemical composition, especially in terms of levels of lipids in the pulp. Fruits with high levels of lipids in the pulp can be important raw material for oil extraction (Tango and Turatti, 1992). However, there is no widespread commercial method for oil recovery from avocado pulp in Brazil. Lucchesi and coworkers,1975 reported that the oil content in avocado pulp can be less than 2% during the first two months of fruit standing on the tree, then increases slowly to the final stage, up fast and can reach 35% of the pulp.

Avocado (*Persea americana Mill*) is an oil-rich, highly nutritious fruit which is abundant locally in Brazil. The avocado oil contains all the beneficial attributes of the fruit, which makes it a very valuable product. It contains high amounts of the anti-cholesterol agent beta-sitosterol, a wide variety of vitamins and antioxidants, and other plant chemicals, which impart beneficial functional properties on humans (Rodríguez-Carpena et al., 2012). The pulp contains 5-30% of the oil (based on fresh weight).

A typical avocado oil is comprised mostly of monounsaturated fatty acids (74%), 11% polyunsaturated fatty acids and about 13% saturated (Arpaia *et al.*, 2006). These percentages vary with cultivars and other influential factors but the oil is very similar to olive oil. It is this high level of monounsaturated fat, which gives the desirable effect of being "anticholesterol" as it prevents the formation of clots the major cause of coronary heart disease. The presence of tocopherols provides antioxidant activity to the oil and combats the lipid oxidation (Dias et al., 2015). Studies on berries and avocado have shown that cultivar and optimal harvesting time are the most important factors determining the amount of fatty acids, triacylglycerol species (TAGs) and antioxidant activity of extracted oil (Ozdemir and Topuz, 2004; Yang and Kallio, 2004).

Description of the studied cultivars: Some important characteristics are described below:

Hass: It is a Guatemalense- mexicanohybrid that belongs to floral group A. The fruit is oval-pyriform, and has a thick and rough peel that confers a good resistance to transport. It weighs from 180 to 300 grams, and the pulp is of good quality and has no fibers (Donadio, 1995).

Breda: it is a Antilhano-Guatemalan hybrid, that belong to floral group A. The fruit is medium size (400-600g), oval shaped, with thin peel and yellow pulp without fibers. The production time is late, varying from June to December. It has a high commercial value, but the production is alternating (COMPANY OF STORES AND GENERAL STORAGE COMPANIES of SÃO PAULO, 2007), cited by Ramos and Sampaio (2008).

Margarida: It is a Antilhano-Guatemalan hybrid, that belong to floral group B. the fruit is spheriod-shaped, and has a thin peel. it weighs 700 grams and low percentage of oil (DONADIO, 1987). The skin is green. The pulp is yellow and without fibers (CEAGESP, 2007), cited by Ramos and Sampaio (2008).to date, there is no investigation on effect of cultivar and ripening stage of Brazilian avocado fruit on oil content, dry matter, and fatty acid composition. Therefore, the objectives of current research were to determine effects of different cultivars and ripening stages of Brazilian avocado fruit (*Persea americana Mill*.) on oil content, dry matter, and fatty acid composition of its avocado oil extracted using soxhlet.

8.2. MATERIALS AND METHODS

8.2.1. MATERIALS

This investigation was carried out at Tsuge Farm,Rio Paranaíba, Minas Gerais State (MG), Brazil. The located at Lat. 19° 25′ 33″S ; Long. 46° 15′ 37″ W. Altitude (11080m over sea level). By Köppen's classification the climate of the region is Aw type in transition to Cwb, with average annual minimum temperature of 11.0 ° C. The mean annual temperature (MAT) averaged 21.1°C, mean annual maximum temperature of 22.3 ° C and total annual precipitation of 2713.65 mm. (PIRES; YAMANISH, 2014).Mature avocado trees on ten to twelve years old of three cultivars; Hass, Breda and Margarida. The trees were spaced 8 m X 6 m; not irrigated and received routine horticultural care (standard pest, disease &fertilizer programs). Avocados are harvested by hand from the trees. The mean yield (17.5/ 30/ 35) tone of fruit per hectare for Hass, Breda and Margarida cultivars respectively.

Three Brazilian avocado cultivars, *Hass, Breda, and Margarida (Persea americana Mill*.), were selected for this experiment. Mature avocado fruits without infection or physical damages were picked up in the second week of June, July, August and September, 2015, according to Tsuge Farm picking standard in Rio Paranaíba city, Minas Gerais State, Brazil. Sufficient fruits were handpicked from the trees; fruits from each cultivar were placed in single layer trays and transported to the laboratory within few hours, and no maturation inhibitor or accelerator was used. Fruits free from any apparent skin damage were selected for analysis.

8.2.2. METHODS

8.2.2.1. Dry matter contents and fruit characteristics:

The pulp was manually removed and homogenized using a domestic blender, dry matter of pulp was determined by drying samples to a constant mass. The peel and seed were separated manually. The malaxed pulp was packaged in polyethylene bags and stored in a refrigerator (4±1°C) until the oil was extract by Soxhlet which was conducted within one day. Fresh weight of fruit , pulp, seed and peel percentage for selected fruits were determined on the samples, which were prepared from six fruits of each cultivar, during four harvesting time.

8.2.2.2. Oil content:

Oil content was determined by Soxhlet extraction, using Hexane as described by IUPAC (1979) method 1.122. Dried ground samples (10g) were placed into the extractor with 250 mL of Hexane and were extracted for 6 hours. After extraction, the samples of mixture Hexane with oil were transferred to a rotary evaporator to separate the hexane from oil at 40 ºC, and percentage of oil content was calculated. Oil content was expressed as both % f.w. and % d.w in pulp. The solvents were of pure grade (purity >97.7%) and were obtained from Merck in Brazil.

8.2.2.3. Fatty acid composition of the extracted oil:

Fatty acid composition of avocado oil was determined as Fatty Acid Methyl Esters (FAMEs) according to the method described by COI/T.20/Doc. no. 24.

8.2.2.4. Statistical analysis:

The obtained data were statistically analyzed using Analysis of variance and Duncan multiple range test and were performed using SAS to test for significance of differences between values at the level of $P < 0.05$. RCB design was used for statistical analysis.

8.3. RESULTS AND DISCUSSION

The data of the physical composition of the avocado fruits of the three cultivars Hass, Breda and Margarida during four harvesting time (June, July, August and September) are presented in Table 14. The mean fruit weight during four harvesting time; the weight of fruits of the Margarida (982.4g) and Breda (627.5g) cultivars were significantly heavier than the fruits of the Hass (272.9g) cultivar. The maximum pulp yield was also obtained for the Margarida cultivar fruits, 83.6%, compared to 70.9% for Breda and around 68.6% for the Hass fruits. The lowest seed content was in Margarida 9.5%, while it was 21.6% in Breda and 19.3% in Hass cultivars. The highest peel content were 12.1% in Hass cultivar, while 7% in Margarida and 7.5% in Breda. Jorge (2014) reported an average weight of (664.51/ 169.16g) for fruits of the Margarida and Hass cultivars, respectively, which were collected in September 2012, from Tsuge farm in São Gotardo city, Minas Gerais State, Brazil. And an average pulp, seed, and peel yield of (72.19/ 64.72%), (12.24/ 14.51%), and (15.57/ 20.77%) in Margarida and Hass cultivar fruits, respectively. While**,** Silva (2011) reported an average weight of (600/ 600/ 200g) for fruits of the Margarida, Breda and Hass cultivars, respectively, which were collected in 2008/2009 and 2009/2010 in August, September from the municipality of Carmo da Cachoeira in the state of Minas Gerais, Brazil. And an average pulp, seed, and peel yield of (72.8/ 69.1/ 59.9%), (15.5/ 17.5/ 25.5%), and (10.5/ 12.6/ 16.7%) in (Margarida, Breda and Hass) cultivar fruits, respectively.

Espinosa-Alonso et al. (2017) studied Six different Mexican genotypes creole avocado (*Persea americana var. drymifolia*) fruits which were collected in July, 2007 from different orchards in Uruapan, Tacambaro, Tancitaro (Michoacan state), Irapuato, and Guanajuato, Mexico. They reported Fresh Hass avocado comprised 74.83±3.52% pulp, 13.06±2.19% seed, and 12.72±1.58% skin based on total fresh avocado weight. The pulp proportion was higher than the 68% of total fresh weight of a ripe Hass avocado grown in New Zealand (Wong et al., 2011). Dry matter content of pulp ranged between 28 and 36% indicating that the fruits were mature; dry matter >21% (in California) and >25% in other countries are considered physiological indicator of fruit maturity (Knight et al., 2002; Pak et al., 2003; Arpaia et al., 2001; Ozdemir and Topuz. 2004). Similar dry matter content has previously been reported for Hass (Hofman et al., 2000; Villa-Rodr_ıguez et al., 2011).

Table 14 shows that the dry matter and the oil content of all cultivars (Hass, Breda and Margarida) of pulp fruits increased from June to September. The dry matter content of pulp increased from (32.93% to 36.24%, from 23.81% to 31.37%, and from 18% to 23%) in rate of (10.1%/ 31.75%/ 27.78%) from June to September in Hass, Breda and Margarida cultivars, respectively. Moreover, the oil amount of fresh pulp similarly increased in the Hass, Breda and Margarida fruits from June to September from (22.38% to 26.04%, from 11.8% to 23.9%, and from 8.04% to 14.37%) in rate of (16.35%/ 102.54%/ 78.73%), respectively. Gaydou et al. (1987) reported that the moisture content of avocado mesocarp decreased steadily with increasing lipid content during the 12–39 weeks after flowering. Ozdemir and Topuz (2004) reported increase in the dry matter contents of Fuerte and Hass cultivars at rate of (25.1/ 43.6%) from November to January, respectively. The oil content increased from November to January at the rates of 23.74% and 77.5% in Fuerte and Hass, respectively. Dry matter and oil content shows variations due to cultivars and harvesting time, which produce moisture losses. Jorge (2014)reported an average of oil content in fresh pulp (8.42%/ 19.71%) and the dry matter (17.01%/ 30.19%) of Margarida and Hass cultivars, respectively. Wear and Silva (2011) reported an average of oil content in fresh pulp (20.9% /12.2%/ 11.9%) and dry matter (38.69%/ 22.43%/ 19.1%) of Hass, Breda, and Margarida cultivars, respectively.

Oil content of avocado pulp varied significantly ($p<0.0001$) among Mexican genotypes and higher than those of Hass. Moreover, Uruapan avocado had significantly lower pulp oil content compared to those grown in other locations. For Tancitaro, the bigger size (BTancitaro) had significantly higher pulp dry matter and oil contents than those from the small fruit (STancitaro). Oil content increased linearly with dry matter content ($Y = 0.9776x - 9.3722$; $r^2 = 0.8568$) consistent with earlier reports of significant correlation between oil content increase and fruit maturity (Paull and Duarte, 2012). This confirms that avocado pulp from Mexican genotypes contain higher oil content than those from Guatemalan, West Indian or their hybrids Varieties (Zafar et al., 2010).

Table 14. Values (mean ± standard deviation) of fruit parameters from '*Hass*', '*Breda'* and '*Margarida'* Brazilian avocado cultivars during different ripening stages.

parameter	Cultivar	Harvesting Time				
		June	July	August	September	Mean
Weight (g)	***Hass***	260.0±20.0b/C	338.3±16.1a/C	218.3±83.9b/C	275.0±43.01b/C	272.9±34.5b/C
	Breda	410.8±70b/B	661.7±107.28a/B	721.7±68.98a/B	715.7±22.95a/B	627.5±60.9a/B
	Margarida	802.5±150a/A	1068.3±32.53a/A	1078.2±132.69a/A	980.5±110.07a/A	982.4±95.3a/A
Pulp (%)	***Hass***	66.4±2.5a/B	68.9±1.9a/C	67.5±5.5a/C	71.6±2.39a/C	68.6±2.5a/B
	Breda	62.9±1.23b/C	75.2±1.34a/B	71.2±5.92a/B	74.4±1.07a/B	70.9±2.1a/B
	Margarida	83.1±1.75b/A	84.8±1.92a/A	84.9±2.01a/A	81.4±2.03b/A	83.6±1.3b/A
Seed (%)	***Hass***	20.5±0.9a/B	18.8±1.6a/A	19.9±5.9a/A	18.1±2.21ª/A	19.3±2.6a/B
	Breda	28.8±1.2a/A	16.7±1.97c/B	21.1±3.83b/A	19.8±1.08b/A	21.6±1.5b/A
	Margarida	9.4±1.45b/C	8.5±1.36b/C	8.6±1.94b/B	11.5±1.53ª/B	9.5±1.2b/C
Peel (%)	***Hass***	13.1±0.8a/A	12.3±2.1a/A	12.6±1.4a/A	10.3±1.31b/A	12.1±0.63a/A
	Breda	8.3±0.99a/B	8.1±1.47a/B	7.7±3.06a/B	5.8±0.19b/C	7.5±0.91a/B
	Margarida	7.5±1.1a/C	6.7±0.61b/C	6.50±0.37b/B	7.1±0.54a/B	7.0±0.53a/B
Pulp dry matter (%)	***Hass***	32.93±0.41b/A	33.3±0.62b/A	34.1±0.81a/A	36.24±1.51a/A	34.1±0.35a/A
	Breda	23.81±0.45c/B	24.63±0.56b/B	31±2.08a/B	31.37±0.78a/B	27.7±0.62d/B
	Margarida	18.0±0.32d/C	19.09±0.61c/C	20.29±0.3b/C	23.0±0.59a/C	20.1±0.21b/C
Pulp oil content (FW) (%)	***Hass***	22.38±0.41c/A	25±0.11b/A	25.6±0.13a/A	26.04±0.19a/A	24.8±0.15b/A
	Breda	11.8±0.35d/B	18.23±0.22c/B	22.11±0.26b/B	23.9±1.03a/B	19.0±0.43c/B
	Margarida	8.04±0.82d/C	12.74±1.19c/C	13.15±0.35b/C	14.37±0.74a/C	12.1±0.65c/C
Pulp oil content (DW) (%)	***Hass***	67.96±1.65c/A	75.1±0.82a/A	75.1±0.93a/A	71.85±1.5b/B	72.5±0.71b/A
	Breda	49.56±0.98d/B	74.0±0.76b/B	71.32±1.2c/B	76.19±0.88a/A	67.8±0.73e/B
	Margarida	44.67±0.69d/C	66.74±0.89a/C	64.81±1.08b/C	62.48±0.87c/C	59.7±0.67e/C

Mean values followed by different uppercase and lowercase indicate significant statistical differences ($p<0.05$) between and within cultivars during harvesting time, respectively, according to the Tukey test.

Major fatty acids of all samples at different ripening stages were presented in Table 15. About 6 FAMEs were identified in the samples. Oleic, palimitic, linoleic and Palmitoleic acids were found to be major fatty acids in all cultivars during ripening stages of which Oleic acid was the highest. Other researchers (Brasil, 1971; Bora et al., 2001; Frega et al., 1990; Gorge, 2014; Martinez Nieto et al., 1988; Medina, 1980; Moreno et al., 2003; Ratovohery et al., 1988; Salgado et al., 2008; Tango et al., 1972, 2004; Villa-Rodríguez et al., 2011; Dreher & Davenport, 2013) have also reported oleic acid as the major fatty acid, followed by palmitic, linolenic, and palmitoleic acids in the pulp of Collinson, Barker, Fortuna, Lula, Bacon, Fuerte, Zutano, Hass, and Margarida cultivars of avocado fruit.

The content of oleic acid as the most important fatty acid in avocado oil varies among different cultivars during ripening stages. The highest amount of oleic acid in all stages of ripening was found for Breda cultivar followed by Margarida and Hass cultivars. Among all of the oil samples oleic acid content were more stable only in Breda cultivar, while it decreased in Margarida and Hass cultivars during ripening stages. In contrast of Palmitoleic acid; The highest amount in all stages of ripening was found for Hass cultivar followed by Breda and Margarida cultivars, which did not agree with the previous study (Ozdemir and Topuz, 2004). The results revealed that the content of palimitic acid as another important fatty acid in avocado oil was decreased at the end of the ripening stage for Breda and Margarida cultivars which were in agreement with (Ozdemir and Topuz, 2004).The highest amount of Palimitic acid in the all stages of ripening was found for Margaridacultivar followed by Hass and Breda cultivars. Linoleic acid content of all samples increased at the end of ripening stage. An inverse relationship was found between the amounts of oleic and linoleic acids in Hass and Margarida cultivars. Gecgel *et al*. (2007) and Zaringhalami *et al*. (2011, 2015), also reported that the lowest content of oleic acid was found in the highest content of linoleic acid in safflower and tea seed oils, respectively.

Table 15. Fatty acid composition (percent) of pulp oils extracted from three different cultivars of Brazilian avocado fruit during ripening stages.

Fatty acids	Cultivars	ripening stages				
		June	July	August	September	mean
C16:0 Palimitic acid	*Hass*	21.18±0.33b/B	21.37±0.23b/B	21.51±0.12b/B	21.84±0.25a/B	21.48±0.13b/B
	Breda	20.95±0.05a/B	20.43±0.03b/C	17.94±0.01d/C	18.04±0.06c/C	19.34±0.03e/C
	Margarida	24.64±0.08a/A	24.7±0.05a/A	23.86±0.07b/A	22.56±0.09c/A	23.94±0.05b/A
C16:1 Palmitoleic acid	*Hass*	12.83±0.07b/A	12.86±0.09b/A	14.80±0.10a/A	12.32±0.08c/A	13.20±0.06d/A
	Breda	4.96±0.01d/B	5.63±0.04b/B	6.55±0.02a/B	5.16±0.02c/B	5.58±0.03b/B
	Margarida	3.43±0.05c/C	3.55±0.02b/C	3.9±0.02a/C	3.1±0.01d/C	3.50±0.03b/C
C18:0 Stearic acid	*Hass*	-	-	0.54±0.01a/A	-	0.14±0.01
	Breda	-	-	-	-	-
	Margarida	-	-	-		-
C18:1 Oleic acid	*Hass*	54.01±0.45a/C	53.22±0.33b/C	52.69±0.23c/C	51.38±0.21d/C	52.83±0.31c/C
	Breda	64.68±0.31d/A	65.27±0.28c/A	65.93±0.25a/A	65.55±0.31b/A	65.36±0.22c/A
	Margarida	59.29±0.23a/B	59.16±0.21a/B	57.18±0.23b/B	55.2±0.28c/B	57.71±0.13d/B
C18:2 Linoleic acid	*Hass*	11.98±0.04b/A	11.86±0.02b/B	10.19±0.07c/B	14.49±0.04a/B	12.13±0.02b/B
	Breda	9.41±0.05b/B	8.29±0.03c/C	9.37±0.02b/C	10.55±0.04a/C	9.41±0.04b/C
	Margarida	11.86±0.03d/A	12.59±0.01c/A	14.58±0.02b/A	17.79±0.05a/A	14.21±0.02e/A
C18:3 Linolenic acid	*Hass*	-	0.69±0.02a/A	0.27±0.01b/A	-	0.24±0.01b/C
	Breda	-	0.38±0.01b/B	0.21±0.01c/B	0.7±0.04a/B	0.32±0.01d/B
	Margarida	0.78±0.02b	-	0.48±0.01c	1.35±0.03a/A	0.65±0.01d/A

ND- Not Identified
Each value in the table represents the mean ±standard deviation. Different uppercase and lowercase indicate significant statistical differences (p<0.05) between and within cultivars during ripening stages, respectively, according to the Tukey test.

The total values of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and unsaturated fatty acids (UFAs) in addition to the MUFAs/SFAs, PUFAs/SFAs, MUFA/PUFA and UFAs/SFAs ratios are presented in table 16.

The monounsaturated fatty acids comprise the largest group in studied avocado oils; the highest mean value of 70.93% achieved in Breda, then 66.03% in Hass which is the principal avocado cultivar grown and accepted by consumers worldwide. While the lowest value in Margarida cultivar was 61.2%.

The pulp oil quality of Breda, Hass and Margarida cultivars studied was found to be better since the SFA values in these pulp oils were 19.34, 21.61,and 23.94% of the total fatty acids, respectively, as well Fortuna and Collinson cultivars were 22.3 and 29.4%, respectively, which reported by Galvão et al, (2014). When compared with the pulp oil of Barker cultivar, which had higher

SFA content, 41.3% of the total fatty acids. The highest mean value of Polyunsaturated acids was in Margarida 14.86%, then 12.37% in Hass, while the lowest value 9.73% was achieved in Breda.

Wood et al. (2003) reported the importance of high ratio of PUFA/SFA to reduce cardiovascular diseases, and they recommended a minimum value of 0.4. Thus, higher values of this index in almost all oils extracted from the pulp of the avocado three cultivars, except for that of the pulp oil of the Collinson cultivar, show the high quality of the oils of the Barker, Collinson, and Fortuna cultivars. The ratio MUFA/PUFA has great importance because of the effects on nutritional properties and oxidative stability of olive oil as in avocado oil. MUFA/PUFA and UFA/SFA showthe ratios of monounsaturated to polyunsaturated fatty acids and unsaturated to saturated fatty acids, respectively. High value of these ratios which indicating high level of Oleic, Palmitoleic acids and low levels of Linoleic, Linolenic, Palmitic or Steraric acids resulted in high stability and nutritional value of oil obtained (Beltran *et al.*, 2004; Desouky *et al.*, 2009; Diraman and Dibeklioglu, 2009). The results revealed that these ratios are related to cultivar and ripening stages of the avocado fruit. In addition, these ratios decreased in last stage of all cultivars.

Table 16. Saturated (SFAs), Monounsaturated (MUFAs), Polyunsaturated (PUFAs), Unsaturated Fatty Acids (UFAs) profile in pulp oils extracted from three different cultivars of Brazilian avocado fruit during ripening stages.

Cultivar	Fatty acids	Harvesting Time				
		June	July	August	September	Mean
Hass	**SFAs**	21.18	21.37	22.05	21.84	21.61
	MUFAs	66.84	66.08	67.49	63.7	66.03
	PUFAs	11.98	12.55	10.46	14.49	12.37
	UFAs	78.82	78.63	77.95	78.19	78.40
	MUFAs/SFAs	3.16	3.09	3.06	2.92	3.06
	PUFAs/SFAs	0.57	0.59	0.47	0.66	0.57
	MUFAs/PUFAs	5.58	5.27	6.45	4.40	5.34
	UFAs/SFAs	3.72	3.68	3.54	3.58	3.63
Breda	**SFAs**	20.95	20.43	17.94	18.04	19.34
	MUFAs	69.64	70.9	72.48	70.71	70.93
	PUFAs	9.41	8.67	9.58	11.25	9.73
	UFAs	79.05	79.57	82.06	81.96	80.66
	MUFAs/SFAs	3.32	3.47	4.04	3.92	3.69
	PUFAs/SFAs	0.45	0.42	0.53	0.62	0.51
	MUFAs/PUFAs	7.40	8.18	7.57	6.29	7.29
	UFAs/SFAs	3.77	3.89	4.57	4.54	4.20
Margarida	**SFAs**	24.64	24.7	23.86	22.56	23.94
	MUFAs	62.72	62.71	61.08	58.3	61.20
	PUFAs	12.64	12.59	15.06	19.14	14.86
	UFAs	75.36	75.30	76.14	77.44	76.06
	MUFAs/SFAs	2.55	2.54	2.56	2.58	2.56
	PUFAs/SFAs	0.51	0.51	0.63	0.85	0.63
	MUFAs/PUFAs	4.96	4.98	4.06	3.05	4.12
	UFAs/SFAs	3.06	3.05	3.19	3.43	3.18

SFAs: Saturated fatty acids (C16:0; C18:0)
MUFAs: Monounsaturated fatty acids (C16:1; C18:1)
PUFAs: Polyunsaturated fatty acids (C18:2; C18:3)

8.4. CONCLUSIONS

The current research provide information about the physical composition of three cultivars of the Brazilian avocado fruits. As well as the dry matter and the oil content of pulp ,and fatty acid composition of that extracted oil, and how they are influenced by ripening stage. Oil content and dry matter differ according to cultivar and ripening stage. The dry matter and oil content were significantly different ($p<0.05$) for all cultivars. The highest valuefor oil content was observed in Hass followed by Breda. The highest percentage of pulp were noticed in Margarida. the highest fruit yield (tone per hectare) were found in Margarida followed by Breda. All selected cultivars are optimal to extract oil because of the high percentage of oil in the pulp, high percentage of pulp in fruits and high yield of fruits. This study shows that Oleic, palimitic, linoleic and Palmitoleic acids were major fatty acids in all ripening stages for all cultivars of which Oleic acid was the highest. Oil content in pulp was lower in June and higher in September for all three cultivars. This concludes that the optimal harvesting time is by waiting until the pulp's oil content increases as described previously. The harvesting time should be achieved from the middle of July in order to obtain the maximum oil yield.

8.5. REFERENCES

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