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Changes in lipid composition during the development of avocado fruits

Mudanças na composição lipídica durante o desenvolvimento de frutos de abacateiro

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The fruits of the avocado tree (*Persea americana* Mill.) have high levels of lipids that can be important raw material for the extraction of oil for the food industry. In addition, avocado has several cultivars with different oil contents. The aim of this study was to evaluate the total lipid content and the fatty acid profile of different avocado cultivars during the maturation period. In this study, Breda, Fortuna, Hass, Margarida, Ouro Verde and Quintal avocado cultivars were used. Oil was obtained by extraction with hot solvent (Soxhlet) and after esterification, samples were injected in gas chromatograph. The highest total lipid contents were obtained by Breda, Hass and Margarida cultivars, with oleic fatty acid showing the greatest amount, ranging from 60% to 66%. Breda, Hass and Margarida cultivars are an alternative to the extraction of oil with great commercial potential to be exploited, avoiding wastes and increasing farmers' income.
Keywords: *Persea americana* Mill., fatty acids, total lipids.

Os frutos do abacateiro (*Persea americana* Mill.) possuem altos teores de lipídios que podem ser uma importante matéria-prima para a extração de óleo para a indústria alimentícia. Além disso, o abacate possui diversas cultivares com diferentes teores de óleo. O objetivo deste trabalho foi avaliar o teor de lipídios totais e o perfil de ácidos graxos de seis cultivares de abacate durante o período de maturação. As cultivares avaliadas foram Breda, Fortuna, Hass, Margarida, Ouro Verde e Quintal. O óleo foi obtido por extração com solvente quente (Soxhlet) e, após esterificação, as amostras foram injetadas em cromatógrafo gasoso. Os maiores teores de lipídios totais foram obtidos pelas cultivares Breda, Hass e Margarida, sendo o ácido graxo oleico o que apresentou a maior quantidade, variando de 60% a 66%. As cultivares Breda, Hass e Margarida são uma alternativa à extração de óleo com grande potencial comercial a ser explorado, evitando desperdícios e aumentando a renda dos agricultores.
Palavras-chave: *Persea americana* Mill., ácidos graxos, lipídios totais.

1. INTRODUCTION

Avocado is considered one of the most important tropical fruits, and in 2021, world production reached 8,685,672.44 tons. In the last five years, an increase of 46.67% and 53.09% in world and Brazilian production was observed, respectively [1]. Its nutritional value and health benefits are among the many factors that have contributed to the increase in fruit production.

The positive effect of avocado on the prevention and treatment of various diseases has been reported by several researchers in recent years, for example, the improvement of hypercholesterolemia, treatment of hypertension, type 2 diabetes mellitus, dyslipidemia, and a significant role in cardiovascular health [2-4]. Extracts with high antioxidant and antimicrobial potential have also been reported [5]. Avocado inhibits the growth of prostate cancer cells [6], induces programmed death of breast cancer cells [7], suppresses hepatic lesions [8], contributes to the treatment of infectious diseases such as HIV [9] and helps in the treatment of osteoarthritis [10].

The broad action of the fruit in relation to human health is related to its composition, that is, its bioactive compounds present mainly in the lipid fraction of the fruit. Avocado oil is rich in chlorophylls (655 mg kg^{-1}), tocopherols: α-tocopherol (18 mg kg^{-1}) and γ-tocopherol

(90 mg kg^{-1}) [11] and phytosterols, mainly represented by β-sitosterol, 251 mg kg-1 of oil [12], in addition to the presence of omega-9 fatty acids and squalene [13].

The lipid fraction of the fruit pulp is mainly composed of saturated fatty acids such as palmitic acid, and monounsaturated fatty acids such as oleic acid [14] and presents low levels of polyunsaturated fatty acids such as linoleic α-linolenic acids [15].

However, the content of bioactive compounds and the lipid fraction, as well as its profile, vary according to cultivar [16, 17], to seasonality and maturation stage. Currently, the fatty acid profile has been studied as possible biomarkers to differentiate areas of avocado cultivation [18], since it is a result of the fruit adaptation to the environment.

In this work, the lipid content and the fatty acid profile of six avocado varieties cultivated in tropical climate were evaluated during the maturation stage.

2. MATERIAL AND MÉTHODS

2.1 Plant material

Avocados used in this experiment were obtained from a commercial avocado orchard located on the borders of BR 381, km 727, municipality of Carmo da Cachoeira, southern region of the State of Minas Gerais, Brazil, with coordinates 21º29'55"S and 45º12'47"W, and average altitude of 987 meters a.s.l. According to the Köeppen classification, the climate of the region is classified as Cwb, mesothermic or altitude tropical, characterized by dry winter, rainy summer and mild temperatures.

Avocado cultivars used in the study are presented in Table 1. Plants belong to an 8-year-old avocado orchard, and plants are arranged in 8 m x 8 m spacing.

For each cultivar, 10 fruits were randomly collected and distributed in five replicates, monthly, during the months of February to September, varying according to the cycle of each cultivar. After harvested, fruits were duly conditioned, identified and sent for evaluation.

Table 1. Floral classification and maturation of avocado cultivars.

Cultivar	Floral Group	Classification regarding maturation period
Breda	A	Late
Fortuna	A	Half season
Hass	A	Half season
Margarida	B	Late
Ouro Verde	A	Late
Quintal	B	Late

2.2 Extraction of total lipids

Lipid extraction was carried out by Soxhlet using ethyl ether P.A. as extraction solvent, according to standards of the Association of Official Analytical Chemists [19]. Initially, samples were dried in an air circulation oven at 65°C until constant mass. After drying, samples were ground, conditioned and stored in freezer until extraction. About 0.5 g of dry sample were weighed, crushed in filter paper cartridge and transferred to the Soxhlet extractor apparatus. Lipids were extracted with ethyl ether P.A. for 4 hours and collected in a flat bottom flask, previously dehumidified in an oven at 105°C and weighed. Then, samples were taken to the oven until complete evaporation of the ether and weighted again. Results were expressed as g 100 g^{-1} of sample, calculated according to equation 1.

$$LIP = ((M-P) / A) * 100 \quad (1)$$

where:
LIP = lipid content (%)
M = mass of the sample plus flask (g)
P = mass of empty flask
A = initial sample

After extraction, composite samples were obtained from the five replicates, which were conditioned in Falcon tube, protected with aluminum foil in order to avoid the degradation of lipids by the incidence of light. These composite samples were kept in freezer for later determination of the fatty acid profile.

2.3 Transesterification of total lipids

Samples were transesterified into methyl esters using potassium hydroxide in methanol and n-hexane, according to AOCS method Ce 2-66 [20].

2.4 Fatty acid profile composition

The fatty acid composition was determined by gas chromatography using a GC-2010 (Shimadzu) chromatograph equipped with a flame ionization detector and a SPTM-2560 capillary column (100 mm x 0.25 mm x 0.2 µm). The standard used was a mixture of 37 methyl esters (Supelco 37 Component FAME Mix). The following operational parameters were used: split injection mode, split ratio 1:100; 1 µL injection volume; 260 ºC detector temperature; 260 ºC injector temperature; oven temperature program: hold at 60 °C for 1 minute, ramp at 4 ºC/min. to 140 ºC, hold for 5 minutes; ramp at 4ºC/min. to 240ºC, hold for 30 minutes. Samples were dissolved in 0.30 mL of n-hexane. Peak identification was resolved by comparing the retention times of the fatty acid methyl ester standards with the retention times of the observed peaks [21].

2.5 Statistical analysis

To compare the difference between percentages of total lipids for the different cultivars during maturation, Tukey test was performed at 5% significance level using the Sisvar 5.6 software [22].

3. RESULTS AND DISCUSSION

3.1 Total lipids

The number of months in which the total lipid content was monitored varied according to the stage of fruit development of each avocado cultivar. Fortuna cultivar showed the fastest development (3 months), whereas Breda cultivar showed more delayed development (8 months), followed of Hass and Margarida cultivars, with 7 months.

The highest total lipid contents at the harvesting point were observed for Hass, Breda and Margarida cultivars, with percentages of 62%, 41% and 39%, respectively. On the other hand, the lowest total lipid contents were observed for Fortuna, Ouro Verde and Quintal cultivars, which varied from 16% to 27%. Despite the low percentage, it is important to consider that there is high yield per area, since avocado is recognized as a highly productive fruit per unit of cultivated area.

Significant difference (Tukey test at 5% probability) was observed for the total lipid content of the different avocado cultivars during fruit development (Figure 1). Fortuna, Hass, Breda and Margarida cultivars presented lipid content at the final development phase greater than or equal to the initial phase, unlike Ouro Verde and Quintal cultivars.

Fortuna cultivar showed total lipid content of 21.58% at the initial development phase, reducing to 14.2% (decrease of approximately 66%), returning to around 20% near harvest. This value differs from that obtained by Massafera et al. (2010) [23] and Oliveira et al. (2013) [24], which detected lipid percentage of 8.50% and 6.43% for the same cultivar, respectively. However, the lipid content in the study was similar to other studies developed with the same cultivar, with contents around 15% and 16% [21, 25].

Ouro Verde cultivar presented initial content of 26.57%, with constant drop until the harvesting period (July), when presented content of 12.7% (reduction of approximately 48%). Similar results were observed by Silva et al. (2014) [26] for avocados grown in the same region, where lipid percentage of 12.3%. In contrast, results identified for other cultivation sites were different, where percentages varied around 8.45% in São Bento do Sapucaí, SP, Brazil [23] and 16.44% in Ribeirão Preto, SP, Brazil [22], showing the strong influence of the environment on lipid production.

The initial phase of Hass cultivar presented values around 46%, followed by a reduction to less than 30% with gradual increase until stabilization in the harvesting period, presenting values around 62%. This behavior is similar to that observed by Villa-Rodríguez et al. (2011) [15], who evaluated the total lipid content of four different maturation stages and found that there is a change in content, but with lower contents compared to the present study, varying from 18% to 20%. According to the author, this low content recorded may be due to the fact that fruits used in this study were already harvested and kept in storage, with inactivation of Acetyl-CoA carboxylase, the key enzyme for the production of long chain fatty acids in fruit tissues. However, the total lipid contents in the present study corroborate with others for the same cultivar, which reached averages between 56% and 65% [27, 28].

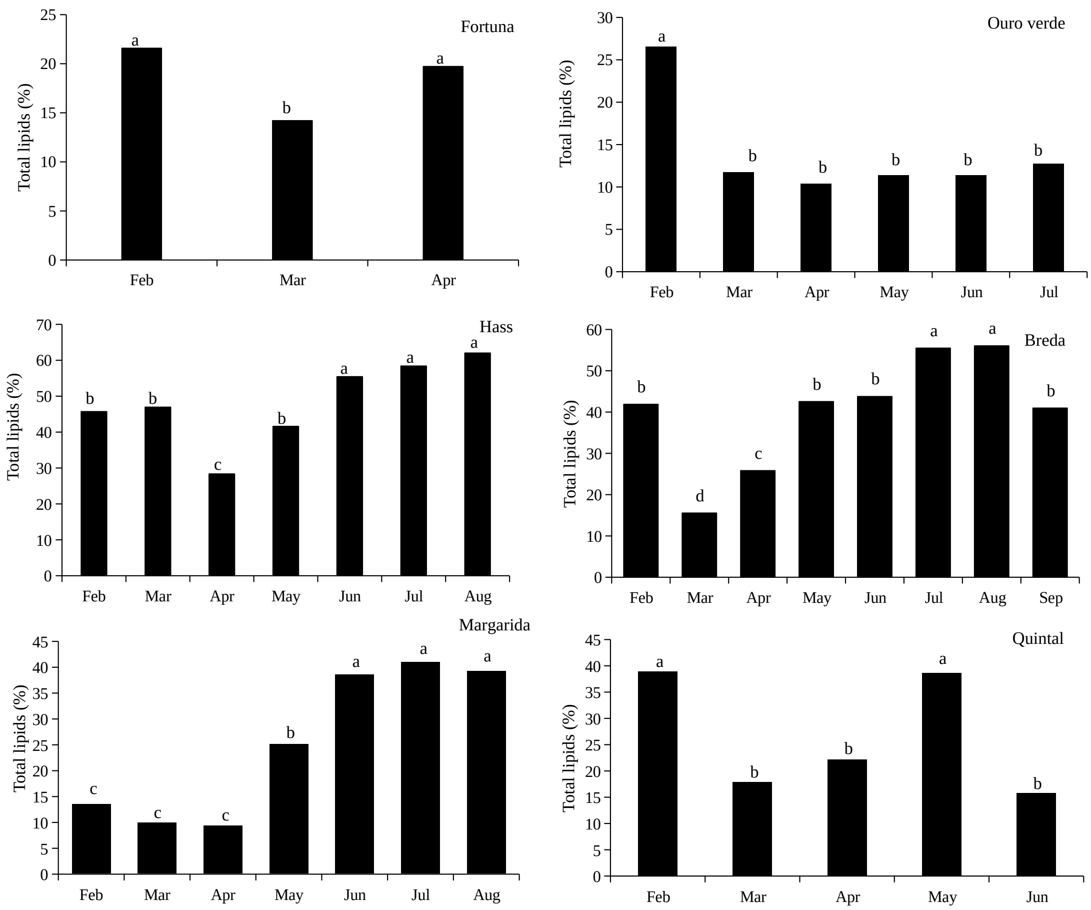

Figure 1. Total lipid content of Fortuna, Ouro Verde, Hass, Breda, Margarida and Quintal avocado cultivars throughout fruit development.

Breda cultivar showed high lipid content at the initial stage of fruit development (41.88%), but it had a sharp drop to contents around 15%, when it gradually increased to about 56% almost at the final stage, when it returned to content of 40.97% at the end of the maturation period. Silva et al. (2014) [26] detected lipid levels for Breda cultivar in the harvesting period around 12.2% for the same region of the present study, which is much lower than values reported. A possible explanation for this large variation may be the climatic variations that occur from year to year and that actively act on the synthesis of lipids, the age of plants (15 years) and the extraction method. However, the values presented are in agreement with the percentage of lipids (47.26%) recorded in avocados from another region [29].

The lipid content of Margarida cultivar was low at the beginning of fruit development (13.54%), increasing gradually until the harvesting period (39.26%), corresponding to an increase in total lipid content three times higher than values observed at the beginning of fruit development. Considering the harvesting point, similar result was found by Jorge et al. (2015) [27], who observed levels of 49.5%, but different from those obtained by Oliveira et al. (2013) [22], Silva et al. (2014) [26] and Salgado et al. (2008) [30], who detected contents of 8.73%; 11.9% and 26.31%, respectively.

Quintal cultivar showed high lipid content at the beginning of fruit development (38.88%), followed by considerable reduction to about 17.9%, increasing again to the initial level, with another drop at the harvesting point to 15.79%. Thus, this cultivar presented low lipid content at the harvesting point, which corroborates other studies, in which mean lipid percentages were 13.6%, 10.95% and 14.7% [22, 25, 29].

3.2 Fatty acids profile

The composition of avocado oil is characterized by high levels of monounsaturated fatty acids (oleic and palmitolytic acids), low content of polyunsaturated fatty acid (linoleic acid), relatively high concentration of saturated fatty acid (palmitic acid) and low content of stearic acid, which is also a saturated fatty acid [31]. In general, saturated fatty acids are harmful to human health, particularly palmitic acid, since they raise LDL cholesterol and the risk of atherosclerosis [32]. However, contrary to the general concept, there are reports that stearic acid does not increase the risk of atherosclerosis and, in fact, reduces LDL cholesterol [32, 33], also reducing blood pressure, improving heart function and reducing the risk of cancer [34-36], resulting in beneficial effects on human health.

As occurred for the total lipid content, alterations in the fatty acid profile were observed along fruit development (Figure 2). In general, palmitic, palmitoleic, oleic and linoleic acids were the most abundant in oils from all cultivars from the beginning of fruit development to the final maturation point. Oleic acid was predominant in the oil from all cultivars, with percentages varying between 60 and 70% at the maturation point, except for Fortuna cultivar, which presented oleic acid content of about 20% at the final maturation stage.

The behavior of Fortuna cultivar differed from the others, presenting reduction of 38% in the amount of oleic fatty acid near the harvesting time. In contrast, an increase of 21% in the amount of linoleic fatty acid was observed. These results differed from those of Massafera et al. (2010) [22], who found higher oleic fatty acid concentration in relation to linoleic acid at the harvesting point of Fortuna avocado grown the region of Ribeirão Preto, SP, Brazil. These results reinforce that avocado is a fruit extremely influenced by the growing conditions.

At the harvesting point, Fortuna cultivar presented lipid profile similar to that of soybean oil, with linoleic acid as the main component, around 33% [37]. The ingestion of linoleic acid has been reported to be of great importance during pregnancy, in the first months after birth, in the elderly and in the treatment of degenerative diseases [38, 39].

During fruit development, Ouro Verde cultivar shows an increase in the oleic acid concentration near the harvesting period, resulting in concentration of 67.5%. There was also a decrease in the linoleic acid (10.4%) and palmitic acid levels (16%), while palmitoleic (5%) and all others (mainly stearic, with concentration of 0.7%) remained stable throughout the fruit development period. Similar results were described by Tango et al. (2004) [31] for Ouro Verde fruits at the harvesting point, where fatty acid concentrations were, 18.3%; 6.8%; 0.5%; 51.9% and 16.9% for palmitic, palmitoleic, stearic, oleic and linoleic acids, respectively.

For Hass cultivar, the most produced worldwide, an increase in the oleic acid concentration in relation to the initial concentration (25.23%) was observed near the harvesting point. Linoleic acid showed a stable behavior throughout the fruit development period, with a slight drop at the harvesting point. Palmitic and palmitoleic fatty acids showed a sharp fall in the period before harvest, increasing again, reaching concentrations of 20.95% and 5%, respectively. Contrary behavior was observed for the other fatty acids, in which maximum concentration was observed in the period before harvest, mainly due to the high levels of margaric (25.18%) and margaroleic (14.25%) fatty acids one month before harvest. Variations in the fatty acid content during fruit maturation were also observed by Villa-Rodríguez et al. (2011) [15] for Hass cultivar. This cultivar was the one with the highest oil content, reaching harvest with concentration of 62%, the majority composed of monounsaturated fatty acids, with emphasis on oleic acid. According to Ferrari (2015) [40], this characteristic allows this cultivar to be a good candidate for oil extraction, since its fatty acid profile qualifies it as a special edible oil, mainly due to the presence of oleic and linoleic acids and to the predominance of monounsaturated fatty acids in its composition (more than 62%).

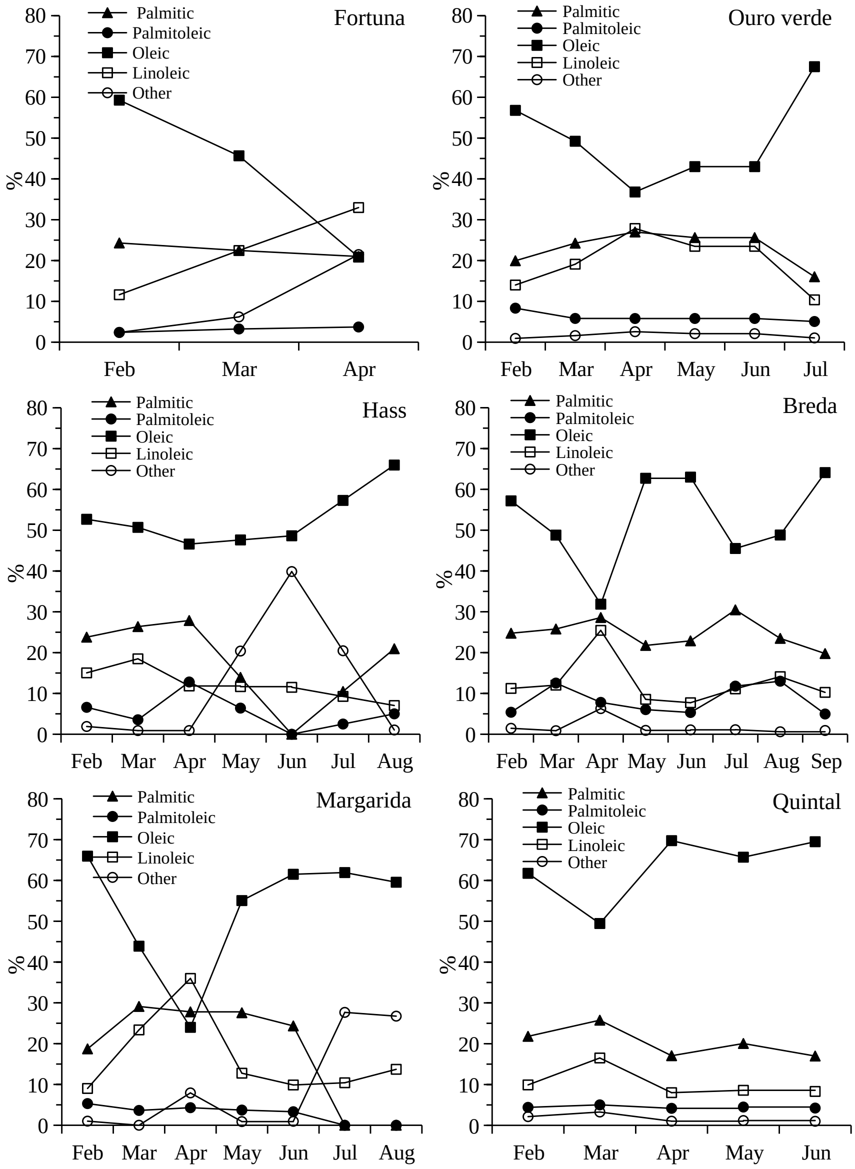

Figure 2. Fatty acid profile of the different avocado cultivars: Fortuna, Ouro Verde, Hass, Breda, Margarida and Quintal throughout fruit development.

Breda cultivar presented the highest oscillation in the oleic fatty acid content during fruit development, presenting an increase in concentration in the period near harvest (64.1%). All other fatty acids present in the cultivar showed a decrease over the period. The contents of the main fatty acids at the end of maturation were similar to those found by Krumreich et al. (2018) [41] in ripe fruits, where the oleic acid content ranged from 57.1% to 64.5%; linoleic (10.5 - 11%), palmitic (19.9% - 21.3%) and palmitoleic (2.7% - 7%) with different extraction methods.

Margarida cultivar showed a sharp drop in the oleic fatty acid concentration at the beginning of fruit development. However, the concentration increased again and remained stable until harvest, with content of 59.55%, representing a 9.70% decrease in the concentration of this acid in relation to the beginning of fruit development. Linoleic acid showed behavior opposite to that of oleic acid. Palmitic and palmitoleic fatty acids were not detected at the harvesting point. In relation to the other acids, high contents were observed at the end of the cycle, mainly represented by margaric acid (22.55%). These results differed from those obtained by Jorge et al. (2015) [27], especially in relation to palmitic and palmitolytic fatty acids, where large concentrations for the same cultivar were observed at the maturation point. The main advantage of this cultivar is the high content of margaric fatty acid, which is saturated and has odd chain. In several studies, saturated fatty acids have been shown to be detrimental to health, in part due to its effect on cholesterol metabolism as well as direct factors associated with the disease. However, margaric fatty acid recently gained research interest mainly due to its beneficial effects on human health, which include strong inverse association with type 2 diabetes, reducing the risk of cardiovascular and coronary heart diseases [42].

For Quintal cultivar, an initial decrease and subsequent increase in the oleic acid content was found, resulting in concentration of 69.49%. The other fatty acids had lower oscillations during fruit development, reaching the harvesting point with concentration of 16.98%; 4.23% and 8.31% for palmitic, palmitoleic and linoleic acid, respectively. These values corroborate those obtained by Tango et al. (2004) [31] for Quintal avocado grown in Campinas, SP, Brazil. This cultivar presents a peculiarity in relation to the other cultivars evaluated in this study: three months before harvest, all fatty acids show stabilization, with minimum oscillations. This characteristic makes the cultivar harvestable before the specified period when fruits are intended for oil extraction, causing the producer to achieve better prices, since about one month before the final stage, the fruit has higher oil content (Figure 1).

A behavior observed in this work was the initial decrease of fatty acid contents, mainly oleic acid in all cultivars. One possible explanation is that this substance is stored in the seed during the initial stage of fruit development. According to Taiz et al. (2017) [43], oils are important forms of reduced carbon storage in many seeds, including those of agronomically important species such as soybean, sunflower, canola, peanut and cotton. Some fruits, such as avocados and olives, also store oils. Reserve lipids contained in plant seeds play an important role in germination, transforming into sucrose for transport in the seedling.

In general, with the exception of Fortuna cultivar, the fatty acid profile is very close to that of olive oil, especially regarding the high levels of oleic acid, since the limits are from 55 to 83% in olive oil [44], making avocado oil an economically and nutritionally viable alternative to olive oil.

4. CONCLUSIONS

Breda, Hass and Margarida cultivars are presented as alternatives for oil extraction due to their high total lipid content.

Of the six cultivars evaluated in this study, Fortuna and Ouro Verde cultivars presented the lowest total lipid content.

There was great variation in the fatty acid composition. Breda, Hass, Margarida, Ouro Verde and Quintal cultivars, with higher levels of unsaturated fatty acids (mainly oleic), are the most suitable for human consumption in relation to cholesterol control.

Quintal cultivar showed stabilization of the fatty acid profile one month before the harvesting point, which makes it an alternative for off-season oil production.

The analyses of the composition of avocado oil obtained in this work confirmed the possibility of using it for human consumption and in the food industry.

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