

Pulp oil from four avocado varieties: extraction, yield, characterization and evaluation of antioxidant activity

Óleo da polpa de quatro variedades de abacate: extração, rendimento, caracterização e avaliação da atividade antioxidante

Aceite de polpa de quatro variedades de aguacate: extracción, rendimiento, caracterización y evaluación de la actividad antioxidante

Received: 09/13/2021 | Reviewed: 09/19/2021 | Accept: 09/22/2021 | Published: 09/25/2021

Danubia Aparecida de Carvalho Selvati Rezende

ORCID: <https://orcid.org/0000-0001-9106-4250>

Federal University of Lavras, Brazil

E-mail: danubiaquimica@hotmail.com

Verônica Andrade dos Santos

ORCID: <https://orcid.org/0000-0002-2032-5780>

Federal University of Lavras, Brazil

E-mail: veronicaandrade@yhoo.com.br

Moacir Pasqual

ORCID: <https://orcid.org/0000-0001-5612-9186>

Federal University of Lavras, Brazil

E-mail: mpasqual@ufla.br

Barbara Sayuri Bellete

ORCID: <https://orcid.org/0000-0001-7063-9449>

Federal University of Lavras, Brazil

E-mail: barbara.bellete@ufla.br

David Lee Nelson

ORCID: <https://orcid.org/0000-0001-7435-3675>

Federal University of Vales do Jequitinhonha e Mucuri, Brazil

E-mail: dnelson@ufmg.br

Gabriela Fontes Alvarenga

ORCID: <https://orcid.org/0000-0002-2064-858X>

Federal University of Lavras, Brazil

E-mail: gabrielafalvarenga@gmail.com

Vanuzia Rodrigues Fernandes Ferreira

ORCID: <https://orcid.org/0000-0002-6974-0846>

Federal University of Lavras, Brazil

E-mail: vanuziarfferreira@gmail.com

Cassia Duarte Oliveira

ORCID: <https://orcid.org/0000-0002-2748-2930>

Federal University of Lavras, Brazil

E-mail: cassiaduartermg2@hotmail.com

Marcus Vinicius Prado Alves

ORCID: <https://orcid.org/0000-0001-5579-8011>

Federal University of Lavras, Brazil

E-mail: ordapsevla@yahoo.com.br

Alex Rodrigues Silva Caetano

ORCID: <https://orcid.org/0000-0003-2561-9646>

Federal University of Lavras, Brazil

E-mail: alexrscaetano@gmail.com

Maria das Graças Cardoso

ORCID: <https://orcid.org/0000-0001-8075-1725>

Federal University of Lavras, Brazil

E-mail: mcardoso@ufla.br

Abstract

The avocado (*Persea americana* Mill.) Is a fruit that is originally from Central America, and it has a high nutritional value. The pulp is the most widely consumed in the form of dessert, salads, sauces and cosmetics. Several studies report that avocado pulp oil has a high concentration of unsaturated fatty acids that provide beneficial health effects. The oil was extracted from four avocado varieties (Quintal, Fortuna, Ouro Verde and Hass) by reflux using two solvents, methanol and hexane, to evaluate the fatty acid profile by GC-FID, as well as the antioxidant potential using

several methods (elimination of DPPH radicals, bleaching of β -carotene, reducing power test and degradation of deoxyribose). The oils had a moderate moisture content, and the most expressive yield was obtained using hexane, mainly for the Quintal and Hass varieties. The percentage of the principal fatty acids varied according to the solvent used, but oleic, palmitic, linoleic, palmitoleic and linolenic acids were identified in all the oils. No antioxidant potential was observed for the oils by the methods used.

Keywords: Natural products; Fatty acids; Biological potential.

Resumo

O abacate (*Persea americana* Mill.) é um fruto originário da América Central e apresenta um alto valor nutritivo, sendo a polpa a parte mais consumida, sob forma de sobremesa, saladas, molhos e cosméticos. Diversos estudos relatam que o óleo da polpa de abacate apresenta um alto teor de ácidos graxos insaturados que proporciona efeitos benéficos a saúde. Este trabalho teve como objetivos analisar o rendimento dos óleos de quatro variedades de abacates (Quintal, Fortuna, Ouro Verde e Hass) extraídos por refluxo utilizando dois solventes, metanol e hexano, avaliar o perfil de ácidos graxos por CG-DIC, bem como o potencial antioxidante frente a diversos métodos (eliminação de radicais DPPH, branqueamento de β -caroteno, ensaio poder redutor e degradação da desoxirribose). Os óleos apresentaram umidade moderada, e rendimento mais expressivo foi para os óleos extraídos em hexano, principalmente para as variedades Quintal e Hass. A porcentagem dos ácidos graxos majoritários variou de acordo com os solventes utilizados, mas, em todos os óleos foram identificados os ácidos oleico, palmítico, linoléico, palmitoleico e linolênico. Os óleos não se mostraram eficazes frente aos métodos utilizados para analisar o potencial antioxidante.

Palavras-chave: Produtos naturais; Ácidos graxos; Potencial biológico.

Resumen

El aguacate (*Persea americana* Mill.) es una fruta originaria de Centroamérica, y tiene un alto valor nutricional. La pulpa es la más consumida en forma de postre, ensaladas, salsas y cosméticos. Varios estudios informan que el aceite de pulpa de aguacate tiene una alta concentración de ácidos grasos insaturados que proporcionan efectos beneficiosos para la salud. El aceite se extrajo de cuatro variedades de aguacate (Quintal, Fortuna, Ouro Verde y Hass) por reflujo utilizando dos solventes, metanol y hexano, para evaluar el perfil de ácidos grasos por GC-FID, así como el potencial antioxidante mediante varios métodos (eliminación de radicales DPPH, blanqueo de β -caroteno, prueba de poder reductor y degradación de desoxirribosa). Los aceites tuvieron un contenido de humedad moderado, y el rendimiento más expresivo se obtuvo con hexano, principalmente para las variedades Quintal y Hass. El porcentaje de los principales ácidos grasos varió según el disolvente utilizado, pero en todos los aceites se identificaron los ácidos oleico, palmítico, linoleico, palmitoleico y linolénico. No se observó potencial antioxidante para los aceites por los métodos utilizados.

Palabras clave: Productos naturales; Ácidos grasos; Potencial biológico.

1. Introduction

Fruits are considered sources of nutritional sources with beneficial properties, and they can also be good sources of natural antioxidants. The avocado is a tropical fruit, grown almost everywhere in the world. The avocado pulp is very energetic and caloric, and it is also a source of monounsaturated fatty acids and antioxidants, so it is very important in human nutrition. However, the chemical composition of the pulp can vary according to its varieties and growing conditions (Green & Wang, 2020; Paramos et al., 2020). According to Chikwendu et al. (2020), avocado pulp is an good source of vitamin D, vitamin E, vitamin B6, vitamin B12 and vitamin C, in addition to containing minerals such as potassium, phosphorus, calcium, iron and sodium. It is also an excellent source of essential amino acids and fatty acids.

Fatty acids are organic acids that differ with respect to the number of carbons and the presence of unsaturation. Several fatty acids are known, such as lauric, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids. The fatty acids commonly found in the avocado pulp oil are oleic (63.73%), linolenic (15.27%), palmitic (14.80%), palmitoleic (4.86%) and linoleic acid (1.09%) (Rydlewsk et al., 2020; Wang et al., 2020).

Oleic acid is most commonly found in avocado pulp. There is evidence that this acid plays an important role in inhibiting the development of cancer cells because of its ability to eliminate the expression of HER2 (erbB-2), an oncogene well known for its involvement in the etiology, progression and metastasis of cancer (Kim et al., 2016; Carrillo, Cavia & Alonso-Torre, 2012).

Fatty acids act on several organs, and their effects on the body have been studied more extensively. Research points to the importance of ingesting essential fatty acids because, in addition to maintaining or improving the cognitive function of patients, these substances can also improve memory, mood and reduce symptoms of depression. However, the absence of this nutrient in the diet increases the risk of dyslexia, attention deficit, depression, dementia and Alzheimer's disease. These substances are closely related to neuronal composition, neurochemical signaling and nerve transmission. Therefore, essential fatty acids contribute to neurotransmission in the nervous system, acting in the modulation of the biophysical properties of the membrane, and pre-synaptic vesicular secretion of neurotransmitters (Acharya et al., 2020; Saini & Keum, 2018). The objectives of this work were to extract the oil from four avocado varieties using methanol and hexane and evaluate the yield and composition of the oils as well as their antioxidant potential.

2. Methodology

The work was developed at the Organic Chemistry Laboratory - Essential Oils and at the CAPQ of the Department of Chemistry (DQI) of the Federal University of Lavras. For this work, only the pulp of four varieties of avocado was used. The avocado varieties came from two regions close to the city of Lavras, Minas Gerais, Brazil: São Tomé das Letras (Quintal and Fortuna varieties) and Carmo da Cachoeira (Ouro verde and Hass varieties).

2.1 Extraction of pulp oils from four avocado varieties

The avocados used were in the final stage of ripening because the fruits have the greatest oil content is at this stage, and they were pulped with the aid of a spoon. The solvents used in this work, methanol and hexane, did not receive any additional treatment.

The oil was extracted according to the method described by Matos (1988) by adding 150 g of the avocado pulp and 300 mL of solvent (methanol or hexane) to a round bottom flask (1000 mL), coupled to a ball condenser. The flask was heated with a heating mantle, and after 6 hours of reflux, the solution contained in the flask was filtered using a Buchner funnel. The solvent was rotary-evaporated, and the sample was stored under refrigeration.).

2.2 Determination of humidity

The pulp moisture was determined by adding 5 g of the pulp and 80 mL of cyclohexane to a round bottom flask (250 mL), which was attached to a Dean stark apparatus. The flask was heated with a heating mantle, and, after 2 hours, the volume of water present in the distillate was measured (Pimentel et al., 2010). The yield of the oil present in the avocado pulp was calculated and expressed in weight of oil per unit weight of material on a dry weight basis.

2.3 Identification and quantification of fatty acids

The identification of fatty acids was accomplished at the CAPQ (Chemical Analysis and Prospecting Center) of the Department of Chemistry at the Federal University of Lavras, where the retention times of injected standards were determined under the same conditions as the sample.

The fatty acid methyl esters were prepared by solubilizing 0.1 grams of sample in 2 mL of hexane and 0.2 mL of 2 mol L⁻¹ methanolic potassium hydroxide solution. This solution was vortexed for 30 seconds and then 3 mL of brine were added. This solutions were left to stand until phase separation and then the supernatant was removed and the determination of fatty acids (FA) was performed on a Shimadzu model CG-17A gas chromatograph using an SP2560 column (100 m long by 0.25 mm internal diameter and 0.2- μ m-thick liquid phase film) and equipped with a flame ionization detector at 260 °C, and a split injector (1:20 ratio) at 260 °C. The initial oven temperature was 140 °C for 5 minutes; the temperature was programmed

to increase at 4 °C per minute to 240 °C, where it was held for 30 min. The total run time was 60 minutes with a linear speed of 20 cm s⁻¹, and 1 µL of sample was injected. The chromatogram obtained by injection of the samples was compared with that obtained from the PUFA standard, and the peaks were integrated. Fatty acids were identified by comparing retention times. The results were presented in percentages of area of the identified fatty acids.

2.4 Antioxidant tests

The evaluation of the antioxidant potential of the avocado pulp oil was achieved at the Laboratory of Organic Chemistry - Essential Oils of the Department of Chemistry at the Federal University of Lavras using the following tests.

2.4.1 Elimination of DPPH radicals

This analysis was performed according to the method described in Rezende et al. (2017), in which an ethanolic solution of DPPH (stock solution) was prepared at a concentration of 40 µg mL⁻¹. To test tubes, 2700 µL of the DPPH stock solution was added, followed by the addition of 300 µL of the essential oil diluted in ethanol in concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL⁻¹. In parallel, the control was prepared, containing all the reagents except oil. After 60 minutes, readings were taken on a spectrophotometer (Shimadzu UV-1601PC) at 515 nm. The percentage of antioxidant activity was calculated using the Equation 1.

$$AA\% = [1 - (A_{\text{sample}}/A_{\text{control}})] \times 100 \quad (1)$$

Where A_{control} represents the absorbance of the negative control (no sample) and A_{sample} is the absorbance of the samples.

2.4.2 β -Carotene bleaching

Oxidation of β -carotene was evaluated according to the method described by Kulisic et al. (2004). In a round-bottom flask (500 mL), 0.003 g of β -carotene, 0.2 g of linoleic acid, 1 g of Tween 20 and 20 mL of chloroform were added. The chloroform was removed using a rotary evaporator at 50 °C, and the residue was dissolved with 500 mL of oxygen-saturated distilled water. To test tubes, 2500 µL of this solution and 100 µL of oil were added at concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL⁻¹, diluted in ethanol. The control was composed only of ethanol. The absorbance was measured immediately on a spectrophotometer (Shimadzu UV-1601PC) at 470 nm. The blank was prepared in the same way as the emulsion, except for β -carotene, which was not added. The synthetic antioxidant used for comparison was BHT (butylhydroxytoluene), and it was prepared in the same concentrations. The tubes were incubated at 50 °C for the oxidation reaction and the absorbance reading was performed after an interval of 60 minutes. The percentage of antioxidant activity (AA%) was calculated using the Equation 2.

$$AA\% = 100 \times [1 - (A_0 - A_t/A_0 - A_{0t})] \quad (2)$$

Where A_0 is the absorbance at the beginning of the incubation and A_t is the absorbance after 60 minutes (both with sample); A_{00} is the absorbance of the control at the beginning of the incubation and A_{0t} is the absorbance after 60 minutes.

2.4.3 Reducing power test

In the evaluation of antioxidant activity using the reducing power method (Silva et al., 2015), 50 µL of the samples in the following concentrations 25, 50, 100, 150, 200, 250 and 500 µg mL⁻¹ were added to 500 µL of 200 mM phosphate buffer, pH = 6, and 500 µL of 1% potassium hexacyanoferrate III. The mixture was stirred and incubated at 50 °C for 20 minutes. Then 500 µL of 10% trichloroacetic acid, 1500 µL of distilled water and 300 µL of 0.1% FeCl₃ were added. The reading was performed using a spectrophotometer (Shimadzu UV-1601PC) at a wavelength of 700 nm. To determine the antioxidant activity, an absorbance curve versus sample concentration was constructed. The positive control used was ascorbic acid.

2.4.4 Degradation of deoxyribose

This method is based on the formation of the hydroxyl radical by means of a Fenton reaction, in which they will attack deoxyribose by degrading it into several fragments, a methodology described by Boulanouar et al. (2013). In test tubes, 100 µL of the sample was added in concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL⁻¹, diluted in water; 100 µL of the FeSO₄ / EDTA mixture, 100 µL of deoxyribose, 700 µL of 0.1 M phosphate buffer (pH = 7.4) and 100 µL of H₂O₂. Then, the tubes were taken to the water bath at 50 ° C, where they remained for 60 min. Then 500 µL of TCA (trichloroacetic acid) and 500 µL of TBA (thiobarbituric acid) were added. After adding the acids, the tubes were boiled for 10 min. The reading was performed at a wavelength of 532 nm immediately after cooling the tubes, in a spectrophotometer (Shimadzu UV-1601PC). Antioxidant activity (AA%) was calculated using the Equation 3.

$$AA\% = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100 \quad (3)$$

Where A_{control} represents the absorbance of the negative control (no sample) and A_{sample} is the absorbance of the samples. The standard used for comparison purposes was mannitol.

3. Results and Discussion (can be separated or together) (TNR font 12 – left aligned)

3.1 Extraction and yield of oils

The oils were extracted using two different solvents, methanol and hexane. The yield values of the pulp oils of 4 avocado varieties are shown in Table 1.

Table 1. Yields of pulp oils from four varieties of avocado: extracted in different solvents.

Avocado Varieties	Solvent (mL)	Mass (g)	Moisture (%)	Oil yields (% p/p BLU)
Quintal	Methanol	169,47		3,57
Quintal	Hexane	169,18	69,72	6,80
Fortuna	Methanol	168,82		3,72
Fortuna	Hexane	169,43	53,43	2,78
Ouro verde	Methanol	154,84		2,49
Ouro verde	Hexane	163,61	46,93	3,30
Hass	Methanol	162,00		3,21
Hass	Hexane	154,60	45,80	6,24

Source: Authors.

According to the data described in Table 1, the oils extracted in hexane had the highest yield, except for the sample of the Fortuna variety, in which the highest yield was obtained with methanol (3.72%). It was also observed that in hexane two varieties showed a higher yield compared to the others (Quintal and Hass) 6.80% and 6.24%, respectively. This result may be related to the type of solvent used, since avocado oil has in its composition several fatty acids (nonpolar compounds) and hexane is an apolar solvent, that is, it will be able to interact with the longest hydrophobic chains of fatty acids and neutral lipids, solubilizing these classes of lipids (Paramos et al., 2020).

The varieties Quintal and Fortuna, from the city of São Tomé das Letras were the ones with the highest percentage of humidity (69.72%; 53.43%) respectively, followed by the varieties Ouro Verde (46.93%) and Hass (45.80%), varieties from Carmo da Cachoeira.

According to Kassim & Workneh (2020) the humidity of the avocado pulp (Hass) was on average 55.00%. This result is above that found in this study, which was 45.80%. The reduction in humidity is associated with increased concentrations of oil and sugars, also indicating greater maturity (Shezi et al., 2020). It is important to note that moisture values for the same avocado species may vary according to the stage of maturation, geographical location of the plant and the appropriate time for harvest (Hernandez et al., 2016).

According to Flores et al. (2019), avocado oil can be used for food purposes, but the high moisture content affects the yield of the lipid content. An alternative to increase this yield would be to dry the pulp before extracting the oil.

The difference in pulp oil yield can be explained according to research by Yang et al. (2020) and Ferreyra et al. (2016), due to the effects caused by harvest season, choice of cultivar, altitude, location and climatic conditions of the place of cultivation, and influence the oil extraction yield. The changes suffered by the fruit during the ripening process can also influence the oil yield, as little oil is found in the avocado pulp, which is still green, showing higher oil concentrations when the fruit is ripe (Shezi et al., 2020).

According to Satriana et al. (2019) there are several solvents that can be used for the extraction of oil from the avocado pulp and the selection influences the extraction yield and composition of the oil, being important the choice of the solvent according to the polarity of the target compounds, the molecular affinity between solute and solvent, mass transfer, the use of a co-solvent, financial viability, environmental safety and human toxicity. In the present work, oil from the avocado pulp methanol and hexane were used, and it was observed that the oil extracted with methanol did not obtain a good yield in relation to hexane, which can be justified by the differences between the polarity of the solvent with the compounds present in the oil.

3.2 Chemical composition of pulp oils from four avocado varieties

Table 2 shows the ratio of fatty acids present in the pulp oils of different avocado varieties.

Table 2. Fatty acid profile of pulp oils from four avocado varieties: extracted in different solvents.

Fatty acid	Composition (%)							
	Quintal		Fortuna		Ouro verde		Hass	
	MET	HEX	MET	HEX	MET	HEX	MET	HEX
Arachidonic	-	-	0,02	-	0,04	-	0,03	0,02
Behenic	0,05	0,04	0,04	0,04	0,04	-	0,04	0,02
Butyric	-	0,04	*	0,03	-	-	0,03	0,03
Docosadienoic	0,05	0,05	0,05	0,06	0,04	-	0,05	0,04
Docosahexaenoic	0,07	-	-	-	-	-	-	-
Eicosanoic	0,17	0,15	0,14	0,14	0,17	-	0,16	0,13
Stearic	0,86	0,93	1,10	1,14	0,64	0,61	1,01	0,52
Heptadecanoic (margaric)	0,07	0,05	0,07	0,07	0,07	-	0,06	0,07
Hexanoic (caproic)	-	0,20	*	0,15	-	-	0,10	0,10
Linolelaid	-	-	-	0,02	-	-	-	-
Linoleic	9,10	9,69	10,86	10,40	9,51	9,45	9,18	11,08
Linolenic	0,69	0,55	0,70	0,63	0,67	0,80	0,56	0,59
Myristic	0,03	0,03	0,03	0,03	0,03	-	0,02	0,03
Octanoic (caprylic)	-	0,04	-	0,02	-	-	0,50	0,35
Oleic	70,17	61,01	67,47	67,78	60,78	67,04	70,07	50,75
Palmitic	15,04	19,06	16,38	17,22	17,15	15,23	16,23	24,62
Palmitoleic	2,80	3,48	1,56	1,54	5,91	6,04	1,80	11,50
Ticosanoic	0,09	0,14	-	-	-	-	-	-

HEX - hexane / MET – methanol. * trace elements. - not found. Source: Authors.

From the data described in Table 2, it can be seen that the varieties under study presented mainly oleic (50.75% to 70.17%), palmitic (24.62% to 15.04%), linoleic acids (11.08% to 9.10%), palmitoleic (11.50% to 1.80%), stearic (1.14% to 0.52%) and linolenic (0.80% to 0.55%), regardless of the solvent used, these results are in line with those obtained by Wang et al. (2020); Rydlewski et al. (2020); Corrales-García et al. (2019); Krumreich et al. (2018) and Tan et al. (2018).

Pulp oils from different avocado varieties are known to be very important sources of oleic acid (Krumreich et al., 2018; Tan et al., 2018). The avocado varieties with the highest percentage of oleic acid were Quintal and Hass (70.17% and 70.07%), both oils extracted with methanol. Only the Fortuna variety showed approximately the same percentage for both solvents (67.47% for methanol and 67.78% for hexane). According to Krumreich et al. (2018) this occurs because the fatty acid content present in the avocado oil is influenced by the type of solvent used in the extraction, the extraction method used, the temperature, pre-treatments and the moisture content of the pulp.

In relation to palmitic acid, the Hass variety stood out from the others, presenting a higher percentage when the oil was extracted with hexane (24.62%). Evaluating the extraction of linoleic acid, it was observed that all oils obtained approximately the same percentage, varying from 9.10 to 11.08%. The Hass variety also stood out in the amount of palmitoleic acid, 11.50% (result obtained from hexane extraction) and the others varied from 1.54 to 6.04%. The amount of stearic acid and linolenic acid found in the varieties under study was low and ranged from 0.52 to 1.14%, both for methanol and for hexane.

Avocado is an important source of fatty acids and the factors that can interfere with nutrient content are the variety, climate and region of cultivation of the avocado, time of the harvest year, maturation stages, among others (Shezi et al., 2020; Yang et al., 2020; Acosta-Díaz et al., 2019; Ferreyra et al. 2016).

Fatty acids are a complex group of compounds that include monounsaturated, polyunsaturated and saturated fatty acids (Wang et al., 2020). The presence in high concentrations of monounsaturated fatty acids such as oleic (omega 9) and

polyunsaturated as linoleic (omega 6) in avocado oil makes it an option for use in culinary preparations and in salads, as they characterize a product with good oxidative stability and high nutritional quality (Tan et al., 2018).

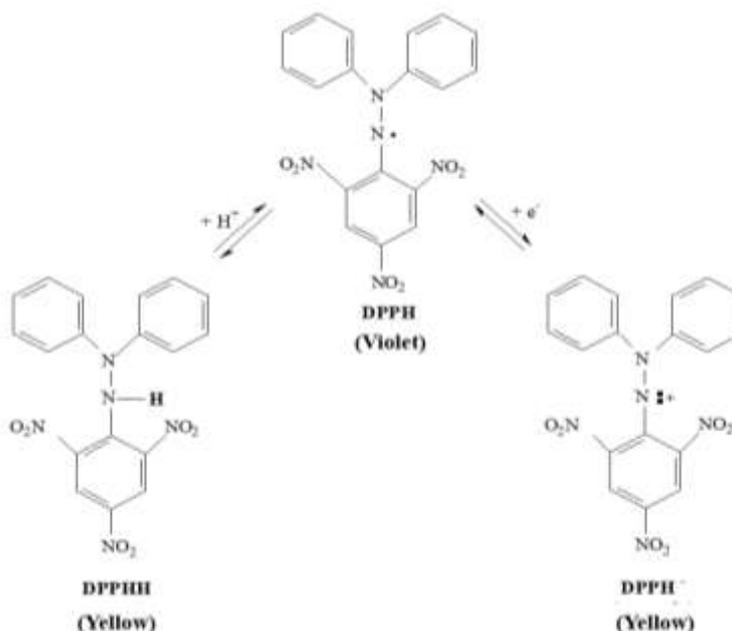
3.3 Antioxidant activity

3.3.1 Elimination of DPPH radicals

The avocado pulp oils under study did not have an antioxidant potential compared to the DPPH radical scavenging method. These data partially corroborate with Daiuto et al. (2014) that analyzing pulp, seed and Hass avocado peel, observed a lower activity for the pulp.

This absence of antioxidant activity of the pulp oils by the method of elimination of the DPPH radical is related to the compounds present in them, since in its constitution no compound was detected that is easy to donate a hydrogen atom or that has a bond double conjugate for DPPH radical stabilization. In addition, according to Sokmen et al. (2020) the nonpolar nature of the oils may explain the low efficiency in stabilizing the hydrophilic DPPH radical. The general mechanism of DPPH reduction can be seen in Figure 1.

Figure 1. General mechanism for reducing DPPH.



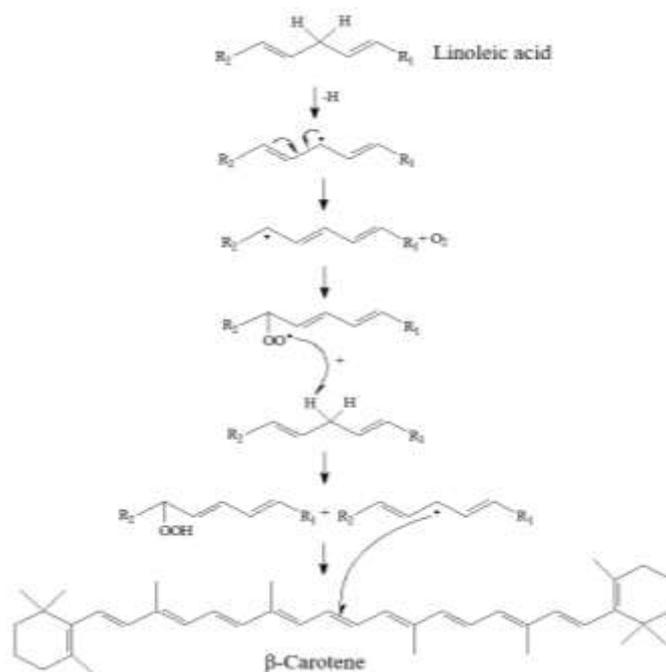
Source: Authors.

It is determined in Figure 1 the possible forms of reduction of DPPH, in which this radical can be stabilized by a hydrogen atom or by an electron (Rezende, 2016). Forero-Doria et al. (2017) obtained good results when analyzing the antioxidant activity of avocado pulp oil, variety Hass, obtained by pressing at 22 °C, with a percentage of elimination of the DPPH radical of 80%. Jimenez et al. (2020) report that there is a great variability in the results of the antioxidant activity of avocado oil, since there are differences in the processes of obtaining the oils, in the avocado varieties, and in the extraction methods, making comparison difficult.

3.3.2 β -carotene bleaching

The oils extracted from the avocado pulps did not have an antioxidant potential compared to the β -carotene bleaching method, a method that assesses the ability of a given substance to prevent oxidation of the β -carotene (Figure 2). This low activity can be explained by the fact that the major constituents of the oils under study are not compounds that contain hydrogen atoms in favorable positions. Phenolic compounds may present better activity in the face of this test, due to the easy removal of a hydrogen atom from these functional groups, by peroxide radicals according to the reagents present in the test (Lekouagheta et al., 2020).

Figure 2. Peroxidation of linoleic acid.

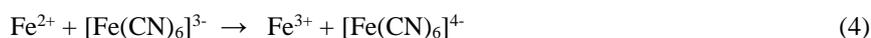


Source: Authors.

Figure 2 shows the oxidation reaction of β -carotene, in which the capacity of the constituents of antioxidant compounds to delay or inhibit the formation of free radicals generated in the peroxidation of linoleic acid is evaluated (Rezende, 2016).

3.3.3 Reducing power test

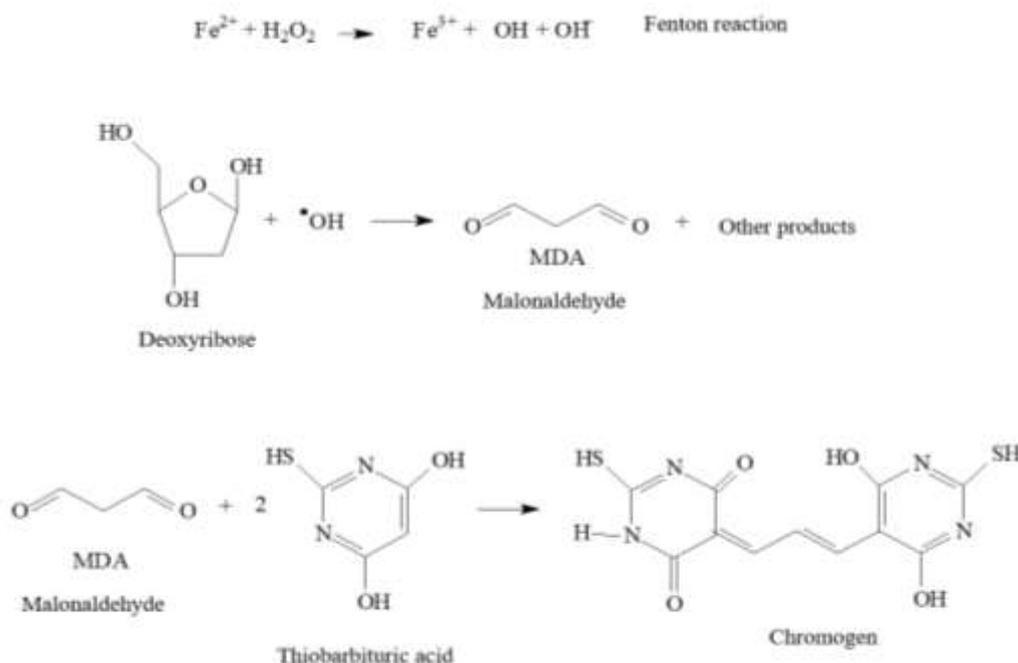
The oils obtained from the avocado pulp did not show antioxidant activity compared to the reducing power method, that is, they did not present in their composition constituents with the potential to reduce iron III $[Fe(CN)_6]^{3-}$ to iron II $[Fe(CN)_6]^{4-}$ (Equation 4). Since this method evaluates the antioxidant capacity of the oil according to the reduction of the ferricyanide ion to ferrocyanide, and as the reduction occurs, the solution tends to become greener.



3.3.4 Degradation of deoxyribose

In this method, the oils obtained from the avocado pulps also did not have antioxidant potential, since the method is based on the formation of the hydroxyl radical by means of a Fenton reaction, in which they will attack deoxyribose degrading it into several fragments, being the main, malonaldehyde (MDA) (Figure 3).

Figure 3. Probable mechanism of formation of the hydroxyl radical through the Fenton reaction and the degradation of deoxyribose by this radical with the formation of the chromogen from malonaldehyde and thiobarbituric acid.

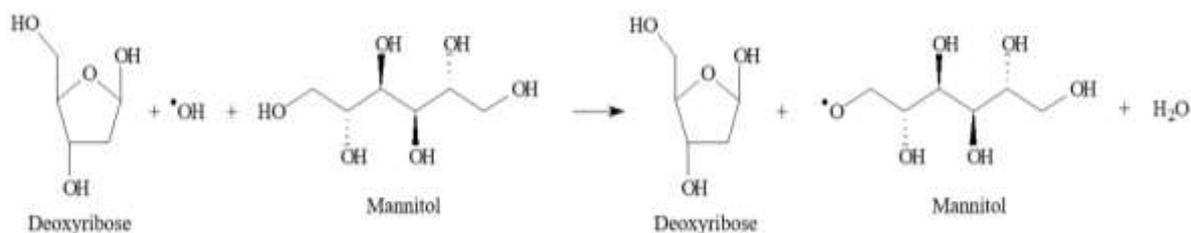


Source: Authors.

Figure 3 shows the likely mechanism of formation of the hydroxyl radical that will attack deoxyribose degrading it into several fragments, one of which is malonaldehyde, which is capable of reacting with TBA (thiobarbituric acid), generating a chromogenic compound that can be quantified by spectrophotometry (Rezende, 2016).

Compounds with antioxidant characteristics such as mannitol (standard) compared to the hydroxyl method have the function of protecting deoxyribose, as there will be a competition for the hydroxyl radical. Then the intensity of the staining will decrease and the formation of malonaldehyde will not occur (Figure 4).

Figure 4. Probable protection from deoxyribose by an antioxidant substance.



Source: Authors.

There are reports in the literature that fruits are rich in compounds that have high antioxidant potential, however, most of these constituents are concentrated in the peels and / or seeds, and these activities are associated with the presence of various compounds, such as vitamins, chlorophyll, and phenolic compounds (Paramos et al., 2020).

The absence of antioxidant activity in the avocado pulp oils used in this work, may be due to the structure of the fatty acids present in the oils, since they do not present structures favorable to a high antioxidant potential, a fact that probably reflects in their high sensitivity to oxidation (Espinosa-Alonso et al., 2017).

García-Vargas and collaborators (2020) obtained ethanolic extract of the avocado peel and kernel of the cultivar Hass through Soxhlet extraction. The moisture of the peel and stone was evaluated, on a fresh mass basis, in addition to the analysis of phenolic compounds and antioxidant tests through the methods of elimination or reduction of the ABTS•+ and Fe³⁺ cation using the equivalent antioxidant capacity of Trolox (TEAC) and tests of the reducing antioxidant power of ferric ion (FRAP). As results, moistures of 71% and 52% were obtained in the bark and stone, respectively. The authors observed a higher content of phenolic compounds in the avocado peel, corroborating the higher antioxidant activity obtained in this residue.

4. Conclusion

The avocado pulp oils under study showed moderate humidity. The most expressive yield was for oils extracted in hexane, which were more relevant for the Quintal and Hass varieties.

The major fatty acids identified in all oils regardless of the solvent were oleic, palmitic, linoleic, palmitoleic and linolenic. The percentage of major fatty acids varied according to the solvents used. Oleic acid for the Quintal and Hass variety showed a higher percentage in the oil extracted in methanol; for the Ouro Verde variety the same was observed in the oil extracted in hexane. Palmitic acid also varied with the solvent, however the Hass variety was the one that showed the greatest difference, with the most relevant value in oil extracted with hexane. It was observed that the percentage of linoleic acid varied only for the Hass variety, with the oil extracted in hexane with a higher percentage. For palmitoleic acid, the oil of the Hass variety extracted in hexane was the one that presented a very expressive percentage in relation to the others.

The oils obtained from the pulps of four varieties of avocados were not effective compared to the methods used to analyze the antioxidant potential.

Studies have shown that monounsaturated and polyunsaturated fatty acids have important physiological functions for the body. They have property against cancer, anti-inflammatory potential and can improve memory, mood and reduce symptoms of depression. However, the absence of fatty acids in the diet increases the risk of dyslexia, attention deficit, depression, dementia and Alzheimer's. The intake of fatty acids is important for actions on neuronal composition, neurochemical signaling and nerve transmission. Fatty acids are able to prevent and / or treat cardiovascular disease, hypertension and atherosclerosis. They also participate in metabolic functions in the body, assisting in the formation of prostaglandins, prostacyclins, thromboxanes and other bioactive compounds responsible for the regulation of cellular functions. However, the avocado pulp oils under study did not show antioxidant potential compared to the methods used. Thus, it is extremely important to use different methodologies to assess antioxidant activity, to try to describe the ability to fight free radicals presented by these metabolites, and thus elucidate the antioxidant mechanisms of different fatty acids on the body.

Acknowledgments

The authors would like to thank the Federal University of Lavras (UFLA), the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), and the Minas Gerais State Research Support Foundation (FAPEMIG) for its financial support and scholarship.

References

- Acharya, P., Uppin, V., Zarei, M., & Talahalli, R. R. (2020). Role of n-3 Fatty Acids on Bile Acid Metabolism and Transport in Dyslipidemia: A Review. *Lipids*.
- Acosta-Díaz, E., Álvarez-Ojeda, M. G., Guzmán-Maldonado, S. H., & Almeyda-León, I. H. (2019). Variability of the total oil content and fatty acid profile of creole avocados from Nuevo Leon, Mexico. *Agronomía Mesoamericana*, 30 (3), 705-719.
- Boulanouar, B., Abdelaziz, G., Aazza, S., Gago, C., & Miguel, M. G. (2013). Antioxidant activities of eight Algerian plant extracts and two essential oils. *Industrial Crops and Products*, 46, 85-96.
- Carrillo Pérez, C., Cavia Camarero, M. D. M., & Alonso de la Torre, S. (2012). Antitumor effect of oleic acid; mechanisms of action. A review. *Nutrición Hospitalaria*, 2012, v. 27, n. 6 (Noviembre-Diciembre), p. 1860-1865.
- Chikwendu, J. N., Udentia, E. A., & Nwakaeme, T. C. (2020). Avocado Pear Pulp (*Persea americana*)-Supplemented Cake Improved Some Serum Lipid Profile and Plasma Protein in Rats. *Journal of Medicinal Food*. 0 (0), 1-6, 2020.
- Corrales-García, J. E., del Rosario García-Mateos, M., Martínez-López, E., Barrientos-Priego, A. F., Ybarra-Moncada, M. C., Ibarra-Estrada, E., & Becerra-Morales, D. (2019). Anthocyanin and oil contents, fatty acids profiles and antioxidant activity of Mexican landrace avocado fruits. *Plant Foods for Human Nutrition*, 74 (2), 210-215.
- Daiuto, É. R., Tremocoldi, M. A., Alencar, S. M. D., Vieites, R. L., & Minarelli, P. H. (2014). Composição química e atividade antioxidante da polpa e resíduos de abacate'Hass'. *Revista Brasileira de Fruticultura*, 36 (2), 417-424.
- Espinosa-Alonso, L. G., Paredes-López, O., Valdez-Morales, M., & Oomah, B. D. (2017). Avocado oil characteristics of Mexican creole genotypes. *European Journal of Lipid Science and Technology*, 119 (10), 1600406.
- Ferreira, R., Sellés, G., Saavedra, J., Ortiz, J., Zúñiga, C., Troncoso, C., & Defilippi, B. G. (2016). Identification of pre-harvest factors that affect fatty acid profiles of avocado fruit (*Persea americana* Mill) cv. 'Hass' at harvest. *South African Journal of Botany*, 104, 15-20.
- Flores, M., Saravia, C., Vergara, C. E., Avila, F., Valdés, H., & Ortiz-Viedma, J. (2019). Avocado oil: Characteristics, properties, and applications. *Molecules*, 24 (11), 2172.
- Forero-Doria, O., García, M. F., Vergara, C. E., & Guzman, L. (2017). Thermal analysis and antioxidant activity of oil extracted from pulp of ripe avocados. *Journal of Thermal Analysis and Calorimetry*, 130 (2), 959-966.
- García-Vargas, M. C., Contreras, M. D. M., Gómez-Cruz, I., Romero-García, J. M., & Castro, E. (2020). Avocado-derived biomass: Chemical composition and antioxidant potential. *Multidisciplinary Digital Publishing Institute Proceedings*, 70, (1), 1-6.
- Green, H. S., & Wang, S. C. (2020). First report on quality and purity evaluations of avocado oil sold in the US. *Food Control*, 107328.
- Hernandez, I., Fuentealba, C., Olaeta, J. A., Lurie, S., Defilippi, B. G., Campos-Vargas, R., & Pedreschi, R. (2016). Factors associated with postharvest ripening heterogeneity of 'Hass' avocados (*Persea americana* Mill). *Fruits*, 71 (5), 259-268.
- Jimenez, P., Garcia, P., Quitral, V., Vasquez, K., Parra-Ruiz, C., Reyes-Farias, M., & Soto-Covasich, J. (2020). Pulp, Leaf, Peel and Seed of Avocado Fruit: A Review of Bioactive Compounds and Healthy Benefits. *Food Reviews International*, 1-37.
- Kassim, A., & Workneh, T. S. (2020). Influence of postharvest treatments and storage conditions on the quality of Hass avocados. *Heliyon*, 6(6), e04234.
- Kim, H. S., Jung, M., Choi, S. K., Moon, W. K., & Kim, S. J. (2016). Different Biological Action of Oleic Acid in ALDH^{high} and ALDH^{low} Subpopulations Separated from Ductal Carcinoma In Situ of Breast Cancer. *Plos one*, 11 (9), e0160835.
- Krumreich, F. D., Borges, C. D., Mendonça, C. R. B., Jansen-Alves, C., & Zambiazzi, R. C. (2018). Bioactive compounds and quality parameters of avocado oil obtained by different processes. *Food chemistry*, 257, 376-381.
- Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food chemistry*, 85 (4), 633-640.
- Lekouaghet, A., Boutefnouchet, A., Bensuici, C., Gali, L., Ghenaïet, K., & Tichati, L. (2020). In vitro evaluation of antioxidant and anti-inflammatory activities of the hydroalcoholic extract and its fractions from *Leuzea conifera* L. roots. *South African Journal of Botany*, 132, 103-107.
- Matos, F. D. A. (1978). *Introdução à fitoquímica experimental*. edições UFC.
- Paramos, P. R., Granjo, J. F., Corazza, M. L., & Matos, H. A. (2020). Extraction of high value products from avocado waste biomass. *The Journal of Supercritical Fluids*, 165, 104988.
- Pimentel, F. A., Cardoso, M. D. G., Batista, L. R., Guimarães, L. G. D. L., & Silva, D. M. (2010). Ação fungitóxica do óleo essencial de *Tanaecium nocturnum* (Barb. Rodr.) Bur. e K. Shum sobre o *Aspergillus flavus* isolado da castanha-do-Brasil (*Bertholletia excelsa*). *Acta Amazonica*, 40 (1), 213-220.
- Rezende, D. A. C. S., Souza, R. V., Magalhães, M. L., Caetano, A. R. S., Carvalho, M. S. S., de Souza, E. C., & das Graças Cardoso, M. (2017). Characterization of the biological potential of the essential oils from five species of medicinal plants. *American Journal of Plant Sciences*, 8 (2), 154-170.
- Rezende, D. A. de C. S. (2016). *Estudo etnobotânico de plantas medicinais de Lavras e região: caracterização química e potencialidades biológicas dos óleos essenciais*. Dissertação (Mestrado em Agroquímica) -Universidade Federal de Lavras, Minas Gerais.

Rydlewski, A. A., Pizzo, J. S., Manin, L. P., Galuch, M. B., Santos, P. D., Zapiello, C., & Visentainer, J. V. (2020). Evaluation of possible fraud in avocado oil-based products from the composition of fatty acids by GC-FID and lipid profile by ESI-MS. *Chemical Papers*, 1-14.

Saini, R. K., & Keum, Y. S. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review. *Life sciences*, 203, 255-267.

Satriana, S., Supardan, M. D., Arpi, N., & Wan Mustapha, W. A. (2019). Development of Methods Used in the Extraction of Avocado Oil. *European Journal of Lipid Science and Technology*, 121 (1), 1800210.

Shezi, S., Magwaza, L. S., Tesfay, S. Z., & Mditshwa, A. (2020). Biochemical changes in response to canopy position of avocado fruit (cv. 'Carmen' and 'Hass') during growth and development and relationship with maturity. *Scientia Horticulturae*, 265, 109227.

Silva, L. F., das Graças Cardoso, M., Batista, L. R., de Souza Gomes, M., Rodrigues, L. M. A., Rezende, D. A. D. C. S., & Nelson, D. L. (2015). Chemical characterization, antibacterial and antioxidant activities of essential oils of *Mentha viridis* L. and *Mentha pulegium* L.(L). *American Journal of Plant Sciences*, 6 (05), 666.

Sokmen, A., Abdel-Baki, A. A. S., Al-Malki, E. S., Al-Quraishy, S., & Abdel-Haleem, H. M. (2020). Constituents of essential oil of *Origanum minutiflorum* and its in vitro antioxidant, scolicidal and anticancer activities. *Journal of King Saud University-Science*, 32, 2377-2382.

Tan, C. X., Chong, G. H., Hamzah, H., & Ghazali, H. M. (2018). Comparison of subcritical CO₂ and ultrasound-assisted aqueous methods with the conventional solvent method in the extraction of avocado oil. *The Journal of Supercritical Fluids*, 135, 45-51.

Wang, M., Yu, P., Chittiboyina, A. G., Chen, D., Zhao, J., Avula, B., & Khan, I. A. (2020). Characterization, Quantification and Quality Assessment of Avocado (*Persea americana* Mill.) Oils. *Molecules*, 25 (6), 1453.

Yang, S., Fullerton, C., Hallett, I., Oh, H. E., Woolf, A. B., & Wong, M. (2020). Effect of Fruit Maturity on Microstructural Changes and Oil Yield during Cold-Pressed Oil Extraction of 'Hass' Avocado. *Journal of the American Oil Chemists' Society*, 97, 779-788.