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Comparative analysis of antioxidant and fatty acid composition in avocado (*Persea americana* Mill.) fruits: Exploring regional and commercial varieties

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A B S T R A C T

On Madeira Island, Portugal, the avocado crop benefits from a Mediterranean climate, exhibiting exceptional phytochemical and biochemical properties. Aiming to evaluate the antioxidant quality and fatty acid composition with a commercial avocado, flours were obtained from five varieties (four regional and one commercial *Hass*) across different tissues (pulp and by-products) and cycles (years and on-tree maturation stages). Results showed that a regional variety with thin purple skin had the highest antioxidant qualities and lipid content, surpassing the other regional and commercial *Hass* varieties. Oleic acid prevailed in all samples, with regional avocados containing arachidonic acid which is an uncommon occurrence among higher plants. Variations in fatty acid content were influenced by the timing of harvest. These outcomes highlight the promising potential of avocados from Madeira Island.

1. Introduction

Avocado (*Persea americana* Mill.) is a popular evergreen tree with a pear-shaped fruit, comprising peel, pulp, and seed. Native to Mexico and Central America, it has global distribution due to its rich nutraceutical content and antioxidant properties (Fan et al., 2022; Vinha et al., 2020). In Europe, Spain leads the avocado production, contributing over 90 %, with Italy, Greece, and Portugal also playing significant roles (Serrano-García et al., 2022). Portugal's main production areas are the Algarve region and Madeira Island (Leça, 2009).

Avocado is considered a “superfood”, because its nutritional and phytochemical properties are often associated to health benefits (Bhuyan et al., 2019). One of the most notable characteristics of the avocado fruit is its oil, which has a high lipid content, predominantly monounsaturated fatty acids. These are associated with numerous health benefits, including those for the cardiovascular systems, associated and evaluated through both atherogenicity (assesses how lipids adhere to cells in the circulatory and immune systems) and thrombogenicity (evaluates the tendency for blood clot formation in vessels) indexes and inflammatory effects (Dreher & Davenport, 2013; Flores et al., 2019).

The high pursuit for avocados and the consequent increase in production and consumption leads to large quantities of by-product waste (primarily peel and seed). This waste amounts to approximately 1.6 million tons of avocado by-products (Salazar-López et al., 2020). It is particularly concerning because these by-products are rich in bioactive compounds, even richer than the pulp, and exhibit antioxidant, antimicrobial, and antimycobacterial properties (Dreher & Davenport, 2013; Sakirigui et al., 2020).

A factor to be considered, which seems to contribute to the final composition of bioactive and phytochemical components of the avocado, is its maturation conditions. Maturation in the tree has been found

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to increase the composition of these components in the pulp (Serrano-García et al., 2022).

In the Madeira Autonomous Region of Portugal, there is a notable lack of studies and information concerning avocado production and analysis. While commercial varieties like *Pinkerton* and *Hass* are known to be in production (Leça, 2009), several regional varieties are also cultivated (Pinheiro de Carvalho et al., 2022). It is essential to undertake a study on these varieties, particularly regarding their nutraceutical and phytochemical components. In 2022, the marketed avocado production in Madeira Island has estimated to approximately 498 tonnes across 51 ha (DRA, 2022). The region boasts considerable agronomic diversity of this crop, attributable to the introduction and seminal propagation of genetic material since the mid-nineteenth century. This includes genetic resources from both the West Indian (*Persea americana* Miller var.

Table 1
Avocado varieties.

Plantation	Varieties	Color	Average Size (cm)	Average Weight (g)	Shape
Regional	 Quebrada Grande (QG)	**Peel** – Green Speckled **Pulp** – Green and Ivory	16	510.49	Rhomboidal
Regional	 Roxa de Casca Fina (RCF)	**Peel** – Purple Speckled **Pulp** – Green and Ivory	23	265.28	High spheroid
Regional	 Roxa de Casca Grossa (RCG)	**Peel** – Purple Speckled **Pulp** – Green and Ivory	20	430.45	Pyriform
Regional	 Cabaça (CB)	**Peel** – Green Yellow Speckled **Pulp** – Light green and Ivory	13	304.40	Narrowly obovate
Commercial	 Hass (HASS)	**Peel** – Green Purple Speckled **Pulp** – Green and Ivory	10	249.24	Ellipsoid

americana) and Mexican (*Persea americana* Miller var. *drymifolia*) genepools (Pinheiro de Carvalho et al., 2022). The surge in commercial avocado production jeopardizes regional varieties, risking agrodiversity, cultural heritage, and nutritional diversity, and with climate change poised to affect water resources for agriculture, it adds even more pressure on local agrosystems for food cultivation (De la Vega-Rivera & Merino-Pérez, 2021).

Given the information presented, it is clear that a comprehensive study on avocado by-products, both nutraceutical and phytochemical, is essential. Furthermore, it is vital to understand the harvest timing – whether at the optimum point or during on-tree maturation – and its impact on the final composition of bioactive products. There is also a need for an in-depth examination of regional varieties in Madeira Island compared with themselves and to commercial ones. This research addresses these issues by examining them across three harvest cycles (representing different years and stages of maturation) specific to Madeira Island regional avocado varieties, focusing on bioactive properties by evaluating antioxidant quality and fatty acid composition and compared them to the most popular commercial variety, *Hass*.

2. Materials and methods

2.1. Chemicals

For antioxidant analysis, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and folin reagents were purchased from Sigma-Aldrich® (Steinheim, Germany). Ethanol was from Aga® (Prior Velho, Portugal). For lipid analysis, the extraction was performed using Petroleum ether from Supelco® (Darmstadt, Germany), and in the GC–MS analyses, pyridine, eicosane, palmitic acid and trimethylsilyl chloride (TMSCl) were obtained from Sigma-Aldrich® (Steinheim, Germany). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) were from Acros Organics® (Waltham, USA).

2.2. Sample harvest and preparation

In this research, were collected five avocado varieties (comprising four regional and one commercial) produced in Madeira Island, Portugal (Table 1).

Three harvest cycles of these varieties were gathered over different years and stages of maturation. The first and second harvest cycles were collected at the optimal harvesting point, at the end of 2020 and beginnings of 2021, followed by at the end of 2021 and beginnings of 2022, respectively. The third harvest cycle represents on-tree maturation, whose harvest has been done one month later than in the second cycle. All the samples were allowed to ripen on a bench.

For each avocado varieties, ten avocados from the same tree were opened, and the pulp, peel, and seed were separately placed into different containers. The pulp was lyophilized at −55 °C using a lyophilizer machine (Labogene, CoolSafe 55–4, Bjarkesvej 5, Denmark), equipped with a vacuum pump (Vacuumbrand, RZ6, Wertheim, Germany), while the peels and seeds were dried in an oven (Heratherm, Thermo-Scientific, Waltham, MA, USA) at 37 °C. After drying, the avocado pulp and peel were ground using a coffee grinder, while the harder seeds were processed with a professional grinder (IKA, Werke M20, Staufen, Germany). The resulting flours were stored in hermetic bags, sealed with a vacuum machine (Audionvac, VMS 153, Derby, UK), and preserved in a refrigerator (Liebherr Profiline, Ochsenhausen, Germany) at −30 °C.

For this work, all samples were analyzed in their dried state, and the flour resulting from the grinding of ten avocados of each variety and their respective tissues, was measured three times for each analysis.

2.3. Determination of antioxidant activity

Antioxidant activity was assessed using a method slightly modified from Fan et al. (2022), incorporating elements from Rocchetti et al. (2019), in which the total phenolic content and antioxidant activity by DPPH radical scavenging assay were determined.

The sample extraction procedure was the same for both methods, where 0.5 g of sample and 5 mL of 70 % ethanol were added to a 10 mL flask and homogenized using a magnetic stirrer (VELP AM4, Usmate (MB), Italy), for 15 min at 1,000 rpm. After that, the mixture was incubated overnight at 4 °C and 120 rpm in an orbital shaker (INNOVA 2100, Edison, New Jersey, USA). Finally, following centrifugation (Eppendorf Centrifuge 5430R, Hamburg, Germany) at 5,000 rpm, at 10 °C for 15 min, the supernatant was stored at −20 °C.

2.3.1. Total phenolic content

To 96-well plates, 25 μL of sample, 25 μL of Folin reagent and 200 μL of ultrapure water were added, followed by an incubation in dark for 5 min. Then, 25 μL of 10 % sodium carbonate was added to the mixture, homogenized, and incubated again in dark for 60 min. Quantification was done using a microplate spectrophotometer (INNO, Dunchon-daero, Jungwon-gu, Seongnam-si, Gyeonggi-do, Republic of Korea) at 765 nm with INNO software, following a gallic acid standard curve (0 to 200 μg/mL) and results expressed in mg GAE/g.

2.3.2. Antioxidant determination by DPPH assay

In 96-well plates, 40 μL of sample and 280 μL of 0.1 mM DPPH methanol reagent were added, followed by an incubation in dark for 30 min. After that, quantification was done using a microplate spectrophotometer (INNO, Dunchon-daero, Jungwon-gu, Seongnam-si, Gyeonggi-do, Republic of Korea) at 517 nm with INNO software, following an ascorbic acid standard curve (0 to 50 μg/mL) and results expressed in mg AAE/g.

2.4. Lipids extraction and quantification

The extraction and determination of lipid content in avocado flours were conducted based on the AACC (2009) method, with a few modifications. In a cellulose thimble, 8 g of avocado pulp was weighed and set in a Soxhlet apparatus. Subsequently, 400 mL of petroleum ether was added to the round bottom flask and also positioned within the apparatus. The extraction process took 4 h, after which the excess solvent was removed using a rotary evaporator (Heidolph, Schwabach, Germany). The lipid content was then transferred to a pre-weighed test tube. To eliminate any remaining solvent, the test tube was placed in an oven at 100 °C, then moved to a desiccator until a constant weight was achieved, concluding with its final weighing.

Quantification was carried out by analyzing the aforementioned extracts using GC–MS. Prior to this analysis, the extracts were derivatized by the silylation method, as described by Rahmouni et al. (2018), with certain modifications. In the test tubes, 5 mg of each extract was combined with 10 μL of the internal standard (IS) solution (eicosane 1 mg/mL), 70 μL of pyridine, 62.5 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and 12.5 μL of trimethylsilyl chloride (TMSCl). This mixture was then incubated in a water bath at 70 °C for 30 min. During this process, the hydroxyl or carboxyl groups in the compounds transformed into trimethylsilyl ethers or esters, respectively. This conversion enhanced the volatility of the compounds, making them more readily detectable in the GC–MS analysis.

GC–MS analyses were conducted using a Shimadzu Gas Chromatograph QP2010 Ultra, complemented by an Autosampler AOC-20i and a High-performance Quadrupole Mass with an electronic impact as the ion source. The separation of compounds took place on a DB-5 J&W capillary column measuring 30 m x 0.25 mm (inner diameter) x 0.25 μm (film thickness), with helium serving as the mobile phase/carried gas at a velocity of 35 cm s^{-1}. The chromatographic conditions were set as follows: an initiation at 6.5 min with a starting temperature of 90 °C sustained for 4 min. This was followed by a temperature ramp of 16 °C min^{-1} up to 180 °C, another ramp of 6 °C min^{-1} to 250 °C, and a final rate of 3 °C min^{-1} to reach 300 °C, which was maintained for 5 min. The injector inlet temperature was set at 320 °C, with the transfer-line temperature at 300 °C and a split ratio of 1:50. The mass spectrometer operated in electron impact (EI) mode, utilizing an energy of 70 eV, scanning within the m/z range of 33–750 at a rate of 1 scan s^{-1}.

From the generated ion chromatograms, peaks were identified by juxtaposing their mass spectra with established mass spectral libraries, namely the Wiley Registry™ of mass Spectral and NIST 14 Mass Spectral data. Calibration curves were derived using five known concentrations, ranging from 5 μg mL^{-1} to 1.5 μg mL^{-1}. The limits of detection (LOD) and quantification (LOQ) were ascertained using the formulas: LOD = (3 x standard deviation) / slope, and LOQ = (10 x standard deviation) / slope. The results were expressed in terms of mg/g of dry weight (DW).

2.4.1. Index of atherogenicity (IA) and index of thrombogenicity (IT)

To assess the nutraceutical value associated with fatty acids, calculations based on Chen & Liu (2020) were performed. These calculations determined the atherogenicity index, which evaluates the lipid adhesion to cells in the circulatory and immunological systems, and the thrombogenicity index, which assesses the propensity to form blood clots in vessels.

$$IA = \frac{[C12:0 + (4 \times C14:0) + C16:0]}{\Sigma UFA}$$

$$IT = \frac{[C14:0 + C16:0 + C18:0]}{[(0.5 \times MUFA) + (0.5 \times \omega 6PUFA) + (3 \times \omega 3PUFA) + \left(\frac{\omega 3}{\omega 6}\right)]}$$

where, UFA are unsaturated fatty acids; MUFA are monounsaturated fatty acids; PUFA are polyunsaturated fatty acids.

2.5. Statistics analysis

The outcomes of each test were presented as mean values (MV) ± standard deviation (SD) based on triplicate measurements for each sample, with analysis conducted using IBM SPSS statistics. The statistical differences among varieties over the cycles and within each variety across cycles were evaluated using Welch ANOVA, followed by post-hoc analysis using Tukey's honestly significant differences. Statistical differences with $p \leq 0.05$ were considered significant.

3. Results and discussion

While avocado pulp is recognized for its nutritional richness and abundant vitamins and phytochemicals that promote health benefits, avocado by-products also possess noteworthy bioactive properties potentially beneficial to human health (Colombo & Papetti, 2019; Fan et al., 2022). To our knowledge, this represents the first study examining antioxidant properties and the fatty acid profile of avocado varieties from Madeira Island. As such, antioxidant assays (Table 2) were conducted to gauge the antioxidant capacity of the pulp, peel, and seed from Madeiran avocado varieties, compared to the most popular commercial variety, across different harvest cycles.

3.1. Total phenolic, antioxidant capacity and lipid content

3.1.1. Total phenolic content (TPC)

Regarding the total phenolic content (TPC), the by-products (peel and seed) generally exhibited higher TPC values (Table 2). In the first harvest cycle, the pulp's TPC ranged between 0.93 and 1.55 mg GAE/g, while the peel ranged between 11.36 and 20.90 mg GAE/g, and the seed revealed the highest phenolic content, varying from 3.73 to 24.41 mg GAE/g. The second harvest cycle showed an enhancement in TPC across all tissues. The peel displayed the highest values, ranging from 22.38 to 46.86 mg GAE/g. This was followed by the seed, ranging from 6.47 to 41.23 mg GAE/g, and lastly, the pulp, from 1.08 to 1.95 mg GAE/g. Finally, the third harvest cycle generally exhibited an upsurge in TPC, with the peel presenting the most considerable values, spanning from 10.98 to 48.47 mg GAE/g. This was followed by the seed, from 4.19 to 33.44 mg GAE /g, and the pulp, from 1.25 to 1.93 mg GAE /g.

Table 2
Total phenolic content (TPC), lipid content estimation, and antioxidant capacity (DPPH assay) in five varieties of avocado.

Antioxidants Assays	Avocado Tissue	Cycles	Varieties QG	RFC	RCG M	CB	HASS
TPC (mg/g GAE)	PULP	1st	1.55 ± 0.04 a	1.09 ± 0.06 a	1.47 ± 0.14 a	0.93 ± 0.00 a	na
		2nd	1.08 ± 0.07 a	1.66 ± 0.01b	1.74 ± 0.04b	1.76 ± 0.08b	1.95 ± 0.02b
		3rd	1.25 ± 0.06 a	1.85 ± 0.03b	1.88 ± 0.01b	1.93 ± 0.06b	1.75 ± 0.05b
	PEEL	1st	20.9 ± 6.04 a	17.94 ± 9.09 ab	11.36 ± 0.93 bc	13.51 ± 6.39c	na
		2nd	33.32 ± 0.28 ab	30.84 ± 0.91 ac	22.38 ± 8.62c	46.86 ± 7.92b	25.08 ± 0.32 ac
		3rd	10.98 ± 2.12 a	48.47 ± 0.37b	32.39 ± 0.61 bc	11.96 ± 9.73 a	35.59 ± 0.36c
	SEED	1st	3.73 ± 0.07 a	21.66 ± 3.53b	21.78 ± 0.55b	24.41 ± 9.74b	na
		2nd	41.23 ± 0.35 a	13.84 ± 7.74b	6.78 ± 7.63c	6.47 ± 0.43c	19.4 ± 1.82b
		3rd	33.44 ± 0.46 a	4.19 ± 3.63b	5.61 ± 3.25b	15.24 ± 4.82c	12.82 ± 1.97c
DPPH (mg/g AAE)	PULP	1st	0.52 ± 0.27 a	0.35 ± 0.04b	0.88 ± 0.26c	0.86 ± 0.40c	na
		2nd	0.43 ± 0.12 ab	0.54 ± 0.06b	0.4 ± 0.21 a	0.67 ± 0.06c	0.42 ± 0.09 ab
		3rd	0.44 ± 0.04 a	0.48 ± 0.17b	0.29 ± 0.18c	0.51 ± 0.12b	0.46 ± 0.12 a
	PEEL	1st	85.65 ± 0.56 a	78.53 ± 3.24 a	90.41 ± 1.43 a	78.43 ± 1.99 a	na
		2nd	90.43 ± 3.87 a	89.42 ± 5.40 a	71.38 ± 2.86 a	87.04 ± 5.50 a	89.35 ± 4.36 a
		3rd	61.84 ± 3.59 a	89.47 ± 2.73b	90.14 ± 0.75b	68.54 ± 1.81 ab	89.62 ± 1.84b
	SEED	1st	9.57 ± 0.50 a	87.29 ± 8.44b	42.18 ± 3.73c	84.24 ± 3.02b	na
		2nd	89.45 ± 5.83 a	78.85 ± 4.46 a	53.82 ± 0.12 a	23.61 ± 0.86 a	88.82 ± 0.13 a
		3rd	88.85 ± 2.84 a	23.44 ± 0.29 bc	14.22 ± 1.31b	53.2 ± 1.05 cd	40.41 ± 1.47 bcd
Lipids (mg/g)	PULP	1st	557.78 ± 5.88 a	730.99 ± 13.86 a	633.16 ± 5.73 a	593.28 ± 11.69 a	na
		2nd	669.52 ± 6.64 a	708.87 ± 5.59 a	379.03 ± 9.39b	368.17 ± 3.75b	628.80 ± 13.15 a
		3rd	635.85 ± 0.75 ad	754.20 ± 11.31b	587.27 ± 10.11 a	413.42 ± 10.39c	698.38 ± 9.86 bd
	PEEL	1st	153.70 ± 1.80 a	276.47 ± 2.97b	155.13 ± 0.07c	75.13 ± 3.82c	na
		2nd	50.22 ± 10.68 ab	78.53 ± 18.74b	24.23 ± 2.70 a	18.81 ± 3.82 a	21.42 ± 2.70 a
		3rd	92.08 ± 0.00 a	127.60 ± 30.05 a	8.81 ± 1.56b	6.17 ± 1.50b	29.50 ± 2.70 bc
	SEED	1st	10.84 ± 2.75 a	43.21 ± 6.70b	7.14 ± 1.50c	8.82 ± 1.56 a	na
		2nd	81.94 ± 3.89 a	24.33 ± 6.91 a	20.33 ± 8.20 a	26.10 ± 2.90 a	14.09 ± 4.85 a
		3rd	27.67 ± 0.00 ab	20.00 ± 2.08 ab	8.27 ± 1.79b	18.90 ± 1.62b	41.24 ± 6.22 a

Values represented as mean ± standard deviation obtained from three tissues (pulp, peel, and seed) in different years and maturation stages (1st, 2nd and 3rd harvest cycle); Values with different letter (a-d) along the row indicates significant statistical differences between varieties (Tukey HSD, $p \leq 0.05$). AAE: ascorbic acid equivalents; GAE: gallic acid equivalents; na- not analysed.

Comparatively, Fan et al., (2022) reported TPC in three rejected avocado pulp varieties, which ranged from 0.15 to 0.21 mg GAE/g, lower than our findings. Similarly, Lyu et al. (2023) documented TPC values in three varieties' pulp, peel, and seed as 0.20 – 0.28 mg GAE/g, 29.22 – 77.85 mg GAE/g and 26.93 – 44.91 mg GAE/g, respectively. These values are lower in relation to pulp, but parallel to our findings concerning the peel and seed. In another study by Wang et al. (2010), TPC from seven different avocado varieties displayed a range from 0.6 mg GAE/g to 4.9 mg GAE/g for the pulp, 4.3 to 13.9 mg GAE/g for the peel, and 19.2 to 51.9 mg GAE/g for the seed. When juxtaposed with our data, their values appear slightly elevated for pulp and seed but diminished concerning the peel. Conversely, Runyogote et al. (2021) reported TPC values for the *Fuerte* avocado seed, ranging from 15.84 to 33.30 mg GAE/g, aligning closely with our observations. Numerous studies have been conducted to determine the phenolic content in avocado and its tissues. Variability in results can arise from several factors, such as ripeness, climate, storage conditions, geographical location, extraction solvents, and lab practices. Given Madeira Island's favorable conductions for avocado cultivation and the regional varieties widespread consumption, it is vital to examine their phenolic content.

3.1.2. Antioxidant capacity (DPPH assay)

Various antioxidant assays gauge the antioxidant capacity of diverse food products (Xiao et al., 2020). The DPPH assay, recognized for its sensitivity and lack for specialized equipment, stands as a prominent method. As a colorimetric technique, DPPH – a stable free radical – undergoes a reduction reaction upon encountering an antioxidant, producing a yellow hue (Higgins et al., 2021; Lyu et al., 2023).

In our study, in the first harvest cycle, the antioxidant capacity of pulp ranged from 0.35 to 0.88 mg AAE/g. Meanwhile, peel and seed values oscillated between 78.43 and 90.41 mg AAE/g and 9.57–87.29 mg AAE/g, respectively. The second harvest cycle manifested a general decrease in pulp values across most varieties, fluctuating between 0.40 and 0.67 mg AAE/g. In contrast, peels displayed a consistent increase, spanning 71.38–90.43 mg AAE/g. Seeds displayed mixed values: two varieties increased, while the others decreased, with values lying between 23.61 and 89.95 mg AAE/g. The third harvest cycle presented a general decline in pulp and seed values for most varieties, with ranges of 0.29–0.51 mg AAE/g and 14.22–88.85 mg AAE/g, respectively. Regional peel varieties displayed mixed trends, but the *Hass* variety ripened on the tree, showed an uptick compared to the traditionally harvested *Hass*.

Comparatively, Fan et al., (2022) reported pulp DPPH assay results between 0.12 and 0.32 mg AAE/g, which are lower than our findings. Lyu et al. (2023) revealed antioxidant potentials of 0.08–0.16 mg AAE/g, 41.53–71.03 mg AAE/g, and 39.36–56.00 mg AAE/g for pulp, peel, and seed, respectively – typically lower than our observations. Conversely, Wang et al., (2010) recorded values surpassing ours in all tissues, suggesting the solvent blend they used might be optimal for assessing antioxidant capacity. As underscored by numerous researchers, antioxidant potential can be influenced by myriad factors, including the choice of solvent.

3.1.3. Lipid content

In our research, the lipid content was ascertained via solvent extraction, employing the Soxhlet method. This method is recognized for its heightened lipid extraction efficiency compared to alternatives like supercritical fluid extraction (Espinosa-Pardo et al., 2020). Among the tissues analyzed, the pulp exhibited the highest lipid concentrations, succeeded by the peel and seed. During the first harvest cycle, the lipid percentages spanned from 55 % to 75 % in pulp, 7.5 % to 27.6 % in peel, and 0.71 % to 4.3 % in seeds. The subsequent harvest cycle revealed a conspicuous decline in lipid amounts for pulp and peel, registering 36.8–70.5 %, and 1.8–7.8 %, respectively. However, the seed lipid content rose to 2.0–8.1 %. The third harvest cycle witnessed an upswing in lipid figures for pulp and peel, achieving 41.3–75.4 % in pulp and 0.6–12.7 % in peel, whereas seed lipid percentages dipped to 0.83–4.1 %.

Remarkably, in this lipid parameter, the *Roxa de Casca Fina* (RCF) variety consistently yielded the most abundant lipid content across all cycles for both pulp and peel, even outpacing the commercial *Hass* variety. Seed lipid dominance varied: the RCF led in the first cycle, *Quebrada Grande* (QG) in the second, and matured in the tree *Hass* in the third.

Contrasting with prior research, Colombo & Papetti (2019) reported seed lipid values of 4.6 % – marginally surpassing our results from first and third harvest cycles, but falling short of our second harvest cycle findings. Their peel values, spanning 4.4–9.1 %, largely align with ours. Another study achieved 2–9 % for peel and 3–15 % for seed, encapsulating our observations, though their seed results often exceeded ours (Salazar-López et al., 2020). In Vinha et al. (2020), both pulp (around 43.5 %) and peel (around 2.2 %) values generally undershot ours, while the seed values, at approximately 14.7 %, exceeded our results. Regarding this study, it is crucial to note that our lipid content, particularly in the pulp, surpasses the values obtained in the other Portuguese region (Algarve) that cultivates avocado fruits. This may underscore the exceptional richness of avocado varieties on Madeira Island. Another study by Daiuto et al., (2014) presented values for pulp, peel, and seed as 64.09 %, 2.18 %, and 3.97 % respectively, which in most instances, paralleled our findings.

3.2. Fatty acid profile

As outlined in the aforementioned methodology, the fatty acid profiles of avocado oil were derived from the petroleum ether extracts using GC–MS. The fatty acids were detected and identified by analyzing their mass spectra and referencing the NIST14 and WILEY 229 mass spectral libraries. The in-depth GC–MS analysis enabled the quantification (in mg of compound/g of oil) of the distinct fatty acids present in the various samples. To our understanding, no previous studies have characterized the fatty acid profiles of the *Persea americana* Mill. species from Madeira Island, making comparative analysis challenging. However, extensive research on commercial varieties provides some benchmarks and guidelines for evaluation.

3.2.1. Pulp

The primary fatty acids detected by GC–MS in the avocado pulp across various varieties and years is displayed in Table 3. The identified saturated fatty acids include palmitic and stearic acids, whereas the major monounsaturated fatty acid is oleic acid. The main polyunsaturated fatty acids detected are linoleic and arachidonic acids. Across all harvest cycles, oleic acid emerged as the predominant fatty acid, corroborating findings from literature (Carvalho & Velásquez, 2015). An exception was observed in the QG variety, in which linoleic slightly surpassed oleic acid. Nonetheless, oleic acid's representation relative to the total fatty acids ranged from 46.9 to 55.9 % across samples. Linoleic acid (16.7–20.5 %) was typically the second most abundant, followed by palmitic acid (3.8—11.4 %), stearic acid (4.7–9.7 %), and arachidonic acid (2.7–5.9 %).

In a study by Galvão et al. (2014), three avocado varieties (*Fortuna*, *Collinson*, *Barker*) were analyzed for their fatty acid profiles across three tissues (pulp, peel and seed). The pulp's average fatty acid percentages aligned closely with our results, with minor deviations. Their oleic acid percentages ranged from 42 to 51 %, palmitic acid values (20–36 %) were notably higher than ours, while linoleic acid (12–19 %) and stearic acid (0.5–2.3 %) mirrored our findings.

Another study investigated the fatty acid profile of three Malaysian avocado varieties alongside the commercial *Hass* variety. The local varieties predominantly contained oleic acid (43.65–51.22 %), followed by palmitic acid (26.41–30.37 %), linoleic acid (12.75–17. 45 %), and stearic acid (0.27–1.56 %). These figures align with our results, except for the heightened palmitic acid levels observed. Meanwhile, the *Hass*

Table 3
Avocado fatty acid composition (mg/g) performed by GC–MS of the pulp of all five varieties.

Cycle	Fatty acid	Varieties QG	RCF	RCG	CB	HASS
1st	Palmitic acid, C16:0	0.50 ± 0.22^{a,AB}	0.60 ± 0.13^{a,AB}	0.68 ± 0.07^{a,A}	1.50 ± 0.27^{b,A}	na
	Oleic acid, C18:1	4.09 ± 0.14^{a,A}	5.28 ± 1.81^{a,A}	3.69 ± 0.77^{a,A}	9.57 ± 1.94^{b,AB}	na
	Linoleic acid, C18:2	5.45 ± 1.07^{a,A}	0.82 ± 0.16^{b,A}	1.13 ± 0.26^{b,A}	1.29 ± 0.31^{b,A}	na
	Stearic acid, C18:0	0.26 ± 0.03^{a,A}	0.49 ± 0.28^{a,A}	0.47 ± 0.27^{a,A}	0.47 ± 0.24^{a,A}	na
	Arachidonic acid, C20:4	0.42 ± 0.14^{a,A}	nd	0.26 ± 0.10^{a,A}	nd	na
	Total	10.7	7.2	6.2	12.8	–
	AI	0.05	0.09	0.13	0.13	–
	TI	0.11	0.32	0.43	0.30	–
2nd	Palmitic acid, C16:0	0.35 ± 0.13^{a,A}	0.23 ± 0.03^{a,A}	1.33 ± 1.24^{a,A}	0.27 ± 0.03^{a,B}	0.32 ± 0.11^{a}
	Oleic acid, C18:1	2.74 ± 0.41^{a,B}	2.33 ± 0.89^{a,A}	24.20 ± 12.35^{b,B}	16.43 ± 7.29^{ab,A}	1.48 ± 0.22^{a}
	Linoleic acid, C18:2	0.63 ± 0.12^{a,B}	1.79 ± 1.11^{a,A}	4.65 ± 3.40^{a,A}	3.29 ± 1.45^{a,A}	1.47 ± 0.15^{a}
	Stearic acid, C18:0	0.19 ± 0.12^{a,A}	1.04 ± 0.24^{a,A}	4.14 ± 2.88^{a,A}	2.06 ± 0.71^{a,B}	nd
	Arachidonic acid, C20:4	0.23 ± 0.02^{a,A}	nd	0.70 ± 0.18^{a,B}	0.42 ± 0.11^{a}	0.25 ± 0.01^{a}
	Total	4.1	5.4	35.0	22.5	3.5
	AI	0.09	0.05	1.17	0.01	0.07
	TI	0.18	0.44	0.92	0.23	0.11
3rd	Palmitic acid, C16:0	0.96 ± 0.14^{ab,B}	0.70 ± 0.20^{a,B}	0.65 ± 0.16^{a,A}	1.56 ± 0.45^{b,A}	0.30 ± 0.10^{a}
	Oleic acid, C18:1	2.36 ± 0.49^{a,B}	5.99 ± 1.47^{b,A}	3.14 ± 0.47^{ac,A}	4.99 ± 0.76^{c,B}	1.53 ± 0.32^{a}
	Linoleic acid, C18:2	0.37 ± 0.11^{a,B}	0.63 ± 0.17^{a,A}	1.31 ± 0.06^{b,A}	1.47 ± 0.31^{b,A}	2.18 ± 0.32^{c}
	Stearic acid, C18:0	0.27 ± 0.13^{a,A}	0.39 ± 0.00^{a,A}	0.50 ± 0.30^{a,A}	0.34 ± 0.11^{a,A}	nd
	Arachidonic acid, C20:4	Nd	nd	0.19 ± 0.00^{a,A}	nd	0.36 ± 0.15^{a}
	Total	4.0	7.7	5.8	8.4	4.4
	AI	0.29	0.10	0.12	0.20	0.06
	TI	0.76	0.30	0.34	0.48	0.10

Values represented as mean ± standard deviation obtained from three tissues (pulp, peel, and seed) in different years and maturation stages (1st, 2nd and 3rd cycle); Values with different letter ($^{a\text{-}d}$) along the row indicates significant statistical differences between varieties (Tukey HSD, $p \leq 0.05$). Different capital letter ($^{A\text{-}B}$) along the column indicates significant statistical differences in same variety and fatty acid, in the distinct harvest cycles (years). AI- Atherogenicity Index; TI- Thrombogenicity Index; na- not analysed; nd- not detected.

variety was rich in oleic acid (62.3 %), succeeded by linoleic acid (15.7 %) and palmitic acid (14.8 %) (Yanty et al., 2011). It is noteworthy that our regional varieties' fatty acid composition mirrors the *Hass* variety's hierarchy, with oleic acid being the most abundant, trailed by linoleic and palmitic acids. Going through the Table 3 by the lines, we can see that in the pulp's first harvest cycle, significant differences were found in relation to oleic and linoleic acids. The CB pulp is enriched in both palmitic and oleic acid, whereas the QG variety has higher amounts of linoleic acid ($p \leq 0.05$). The fatty acid composition of second harvest cycle pulp is quite similar among avocado varieties. Oleic acid, however, emerges as an exception. While no distinctions among QG, RCF, and *Hass* varieties were detected, when comparing these three cultivars to RCG, notable differences become apparent. This variety accumulates 16 times more oleic acid than the commercial one. The CB variety, with intermediate level of oleic acid, does not differ from the remaining varieties ($p > 0.05$). Third harvest cycle pulp displays significant variations in the palmitic, oleic and linoleic acids content. Specifically, for palmitic acid, CB differs from nearly all varieties except QG, which remains similar to the other samples. QG and *Hass* contain similar amounts of oleic acid and the lowest of the studied samples, while RCF presents the highest values of this compound ($p > 0.05$). The CB pulp, despite accumulating less oleic acid than RCF, is enriched in this unsaturated fatty acid, compared to the QG and *Hass* varieties. *Hass* avocados have the most enriched pulp in linoleic acid ($p \leq 0.05$). When comparing regional varieties, RCG and CB pulps are also enriched in this PUFA ($p \leq 0.05$), whereas QG and RCF varieties have the lowest levels.

A statistical analysis (Table 3) ($p \leq 0.05$) using capital letters (column-wise) for fatty acids across different avocado varieties indicates harvest cycle-dependent variances. Notably, in the QG variety, oleic and linoleic acids levels are significantly higher in the first harvest cycle, while palmitic acid rises from the second to the third harvest cycles. Stearic and arachidonic acids display no significant inter-cycle disparities. For the RCF variety, oleic, linoleic, and stearic acids remain steady across harvest cycles, while palmitic acid varies between the second and third harvest cycles, with the decreased and increased, respectively. The second harvest cycle pulp of the RCG variety is enriched in oleic and arachidonic acids. CB variety's palmitic and stearic acids presents inter-cycle variations, while the oleic acid content differs in the second and third harvest cycles. Linoleic acid levels, however, remain consistent throughout the three harvest cycles. As arachidonic acid appears only once in the cycle, an analysis remains inconclusive.

3.2.2. Peel

Regarding the peel (Table 4), oleic acid was again predominant, with values across cycles ranging from 47.7 to 62.3 %. It was followed by linoleic acid (15.6–30.3 %), stearic acid (11.3–17.5 %), palmitic acid (1.2–7.2 %), and arachidonic acid (3.3–5.3 %). A prior study examining three avocado varieties across all tissues reported fatty acid values of 39.9 to 43.01 % for oleic acid, 17 to 22.6 % for linoleic acid, and 19.79 to 28.93 % for palmitic acid. These findings are comparable to ours, though therés a slight bias towards our results. The only exception is the palmitic acid, which we observed in lower quantities (Galvão et al., 2014). Another study by Takenaga et al. (2008) analyzed the fatty acid composition in the peel and seed of commercial avocado varieties – *Hass*, *Fuerte* and *Bacon*. They recorded values in the peel ranging from 45.9 to 58.2 % for oleic acid, 10.7 to 12.5 % for linoleic acid, 18.6 to 20.0 % for palmitic acid, and mere 0.1 % for stearic acid. These results differ from ours, with only their palmitic acid levels exceeding ours. This distinction is noteworthy since our findings show higher values in regional varieties, while the aforementioned studies focus on commercial varieties known and studied globally.

Upon analyzing the Table 4 row-by-row, the first peel harvest cycle displayed significant differences ($p \leq 0.05$) in linoleic acid among the varieties. QG differed from all other varieties, mirroring observations from the pulp cycle. Stearic acid also manifested significant differences between QG and RCG, but the rest were analogous. In the second harvest cycle, a divergence from the pulp pattern was seen, with noticeable differences in all acids except linoleic. Considering palmitic acid, the highest levels were found in the *Hass* peel. Considering the regional varieties, RCG is enriched in palmitic acid only in comparison to RCF. The CB variety is enriched in oleic acid, thus differing from all the other avocado varieties. This variety is also enriched in stearic acid in comparison to the RCF and *Hass* varieties. The QG and RCG stearic acid content does not differ from any of the other varieties. Arachidonic acid

Table 4
Avocado fatty acid composition (mg/g) performed by GC–MS of the peel of all five varieties.

Cycle	Fatty acid	Varieties QG	RCF	RCG	CB	HASS
1st	Palmitic acid, C16:0	0.32 ± 0.05 ^{a,A}	0.23 ± 0.02 ^{a,A}	0.30 ± 0.06 ^{a,A}	0.24 ± 0.02 ^{a,A}	na
	Oleic acid, C18:1	2.31 ± 0.40 ^{a,A}	3.07 ± 2.46 ^{a,A}	2.28 ± 0.30 ^{a,A}	4.22 ± 2.27 ^{a,A}	na
	Linoleic acid, C18:2	3.89 ± 1.76 ^{a,A}	0.68 ± 0.22^{b,A}	0.70 ± 0.27^{b,A}	0.72 ± 0.30^{b,A}	na
	Stearic acid, C18:0	0.55 ± 0.08 ^{a,A}	0.83 ± 0.52 ^{ab,A}	1.45 ± 0.37^{b,A}	1.13 ± 0.34 ^{ab,A}	na
	Arachidonic acid, C20:4	0.39 ± 0.07 ^{a,AB}	0.27 ± 0.05 ^{a,A}	0.29 ± 0.09 ^{a,A}	0.39 ± 0.39 ^{a,A}	na
	Total	7.5	5.1	5.0	6.7	–
	AI	0.05	0.05	0.08	0.19	–
	TI	0.27	0.33	0.67	0.41	–
2nd	Palmitic acid, C16:0	0.29 ± 0.05 ^{ab,A}	0.25 ± 0.01 ^{a,A}	0.40 ± 0.05^{b,A}	0.26 ± 0.02 ^{ab,A}	0.92 ± 0.09^{c}
	Oleic acid, C18:1	10.69 ± 2.41 ^{a,B}	11.84 ± 2.07 ^{a,B}	11.62 ± 2.61 ^{a,B}	27.58 ± 6.51^{b,B}	2.88 ± 0.34 ^{a}
	Linoleic acid, C18:2	0.86 ± 0.20 ^{a,B}	2.56 ± 1.28 ^{a,A}	1.97 ± 1.40 ^{a,A}	1.38 ± 0.38 ^{a,A}	0.74 ± 0.12 ^{a}
	Stearic acid, C18:0	3.67 ± 1.86 ^{ab,B}	1.83 ± 0.19 ^{a,B}	4.50 ± 2.11 ^{ab,A}	7.26 ± 2.30^{b,B}	0.22 ± 0.01 ^{a}
	Arachidonic acid, C20:4	0.71 ± 0.25 ^{ab,A}	0.57 ± 0.05 ^{a,B}	1.43 ± 0.38^{b,B}	0.84 ± 0.17 ^{ab,B}	nd
	Total	16.2	17.1	19.9	37.3	4.8
	AI	0.60	0.02	0.03	0.01	0.22
	TI	0.67	0.22	0.65	0.50	0.39
3rd	Palmitic acid, C16:0	0.30 ± 0.05 ^{a,A}	0.27 ± 0.03 ^{a,A}	0.36 ± 0.04 ^{a,A}	0.31 ± 0.04 ^{a,A}	0.55 ± 0.02^{c}
	Oleic acid, C18:1	6.78 ± 2.71 ^{a,AB}	5.74 ± 0.89 ^{a,A}	4.11 ± 0.97 ^{a,A}	27.81 ± 4.43^{b,B}	1.43 ± 0.06 ^{a}
	Linoleic acid, C18:2	0.41 ± 0.21 ^{a,B}	2.19 ± 1.23 ^{a,A}	1.75 ± 0.76 ^{a,A}	6.67 ± 6.30 ^{a,A}	0.55 ± 0.13 ^{a}
	Stearic acid, C18:0	1.72 ± 0.54 ^{ab,AB}	1.24 ± 0.43 ^{a,AB}	1.61 ± 0.11 ^{ab,A}	3.53 ± 1.26^{b,AB}	0.22 ± 0.01 ^{a}
	Arachidonic acid, C20:4	0.28 ± 0.05 ^{a,B}	nd	0.32 ± 0.02 ^{a,A}	0.36 ± 0.07 ^{a,A}	0.21 ± 0.00 ^{a}
	Total	9.5	9.4	8.1	38.7	3.0
	AI	0.04	0.03	0.05	0.01	0.52
	TI	0.40	0.31	0.47	0.22	0.45

Values represented as mean ± standard deviation obtained from three tissues (pulp, peel, and seed) in different years and maturation stages (1st, 2nd and 3rd cycle); Values with different letter ($^{a-d}$) along the row indicates significant statistical differences between varieties (Tukey HSD, $p \leq 0.05$). Different capital letter ($^{A-B}$) along the column indicates significant statistical differences in same variety and fatty acid, in the distinct harvest cycles (years). AI- Atherogenicity Index; TI- Thrombogenicity Index; na- not analysed; Nd- not detected.

was not detected in the *Hass* peel. The content in this PUFA is significantly higher in the RCG peel compared to the RCF variety. However, comparing these two varieties to QG and RCF, we found no significant differences.

By the third peel harvest cycle, palmitic, oleic, and stearic acids all demonstrated noteworthy variations. Similarly, to the second cycle, the *Hass* variety differs from all the others considering its enrichment in palmitic acid, although no differences were detected between regional varieties. A remarkable increase in oleic acid was found in the CB peel compared to all other samples, but especially compared to the *Hass* variety. The content of stearic acid is significantly higher in the CB peel, compared to the peel of the *Hass* and the RCF samples. QG and RCG stearic acid levels do not differ from the other samples. All varieties have similar levels of arachidonic acid but was not detected in the RCF peel.

The statistical analysis ($p \leq 0.05$) when observed column-wise reveals unique patterns across the various avocado varieties and harvest cycles for distinct fatty acids. Palmitic acid displayed consistency across all varieties and cycles, albeit with minor value alterations. Oleic acid exhibited variations based on cycles; QG differed between the first and second harvest cycles, while RCF, RCG, and CB each had a unique second harvest cycle. Linoleic acid's variation was exclusive to QG. Stearic acid showcased variations in QG, RCF, and CB, but maintained consistency between the first and second harvest cycles. Arachidonic acid displayed variations across all varieties, possibly stemming from fluctuating quantities. Broadly speaking, the second harvest cycle of the peel distinctly diverges from the other cycles in terms of its differences.

3.2.3. Seed

For seeds, as illustrated in Table 5, the predominant fatty acid is oleic acid, with concentrations ranging between 54.5 and 69.4 %. It is trailed by linoleic acid (14.3–17.3 %), stearic acid (7.4–13.1 %), palmitic acid (1.8–11.8 %), and finally arachidonic acid (1.6–3 %). Takenaga et al. (2008) explored the lipid and fatty acid composition in the pulp and seed of three avocado varieties. Their findings diverge from ours, both in the actual percentages and in the ranking of fatty acids quantity. In their study, linoleic acid emerged as the most abundant, with values between 35.3 and 38.2 %, succeeded by oleic acid (22.4–24.1 %), palmitic acid (17.7–19.0 %) and stearic acid (0.3–0.8 %). Another research by Galvão et al. (2014) analyzed the fatty acid profiles of three avocado varieties across pulp, peel, and seed. Their results slightly differ from ours. Specifically, in the seed, linoleic acid was predominant with values spanning 23.95 to 29.38 %, followed by palmitic acid (12.64–22.41 %), oleic acid (10.88–17.59 %), and stearic acid ranging between 0.94 and 2 %. Both studies noted higher levels of ω-6 fatty acids in seeds but lower value in ω-9 fatty acid when contrasted with our findings.

Analyzing the seeds in a manner similar to the pulp and peel, and the lowercase letters along the rows, we find that some significant differences were found ($p \leq 0.05$) between the varieties in the first harvest cycle. The RCG variety is enriched in linoleic acid and stearic acids compared to all the other varieties.

Concerning the second harvest cycle, differences were only observed in the oleic acid content. The QG variety seed contain more oleic acid than all the other varieties, except for the *Hass* samples. The lowest amount of this MUFA was found in CB seeds. The amount of this acid in RCF and RCG varieties does not significantly differ from either CB or *Hass* seeds.

In the third harvest cycle, variations between varieties are evident only for oleic acid. Here, the QG and CB varieties are both equal and significantly enriched in oleic acid relatively to the other two varieties, being the RCF and RCG identical.

Examining the data column-wise (Table 5) using the capital letters, for the QG variety, the high variability in values is evident. There is a clear difference between the oleic acid values from the first harvest cycle and the remaining two harvest cycles. As for linoleic acid, the increase in this compound separates the third harvest cycle from the others. Considering stearic acid levels, the first and third harvest cycles differ from each other, with the second harvest cycle resembling the others. Among the other varieties, variations are detected in just one fatty acid for each. Specifically, in RCF variety, the second harvest cycle varies from the others of oleic acid. For RCG, the first and third harvest cycles differ from each other, with the second harvest cycle aligning with them in linoleic acid. In the CB variety, variations are seen in stearic acid, where the first harvest cycle diverges from the subsequent cycles.

3.3. Relationships among antioxidants, total and unsaturated fatty acids

A correlation (Table 6) was conducted to explore the relationship between antioxidants (DPPH and TPC) and both total and unsaturated

Table 5
Avocado fatty acid composition (mg/g) performed by GC–MS of the seed of all five varieties.

		Varieties				
Cycle	Fatty acid	QG	RCF	RCG	CB	HASS
1st	Palmitic acid, C16:0	0.30 ± 0.03 ^{a,A}	0.29 ± 0.03 ^{a,A}	0.38 ± 0.21 ^{a,A}	0.37 ± 0.04 ^{a,A}	na
	Oleic acid, C18:1	4.15 ± 2.08 ^{a,A}	3.69 ± 1.31 ^{a,A}	12.57 ± 6.28 ^{a,A}	12.89 ± 3.68 ^{a,A}	na
	Linoleic acid, C18:2	0.63 ± 0.32 ^{a,A}	1.56 ± 0.88 ^{a,A}	4.05 ± 1.05^{b,A}	1.56 ± 0.41 ^{a,A}	na
	Stearic acid, C18:0	0.74 ± 0.29 ^{a,A}	0.78 ± 0.22 ^{a,A}	2.55 ± 1.33 ^{ab,A}	3.67 ± 1.05^{b,A}	na
	Arachidonic acid, C20:4	Nd	0.25 ± 0.02 ^{a,A}	0.56 ± 0.10 ^{a,A}	0.58 ± 0.13 ^{a,A}	na
	Total	5.8	6.6	20.1	19.1	na
	AI	0.06	0.05	0.02	0.02	na
	TI	0.85	0.30	0.21	0.53	na
2nd	Palmitic acid, C16:0	Nd	0.28 ± 0.02 ^{a,A}	nd	0.28 ± 0.00 ^{a,A}	0.36 ± 0.11 ^{a}
	Oleic acid, C18:1	31.21 ± 7.94 ^{a,B}	11.72 ± 3.19 ^{bc,B}	17.33 ± 1.80 ^{bc,A}	7.17 ± 1.24 ^{cA}	23.44 ± 7.13^{ab}
	Linoleic acid, C18:2	2.20 ± 0.80 ^{a,A}	2.56 ± 2.97 ^{a,A}	2.37 ± 0.07 ^{a,AB}	3.92 ± 0.95 ^{a,A}	2.76 ± 1.96 ^{a}
	Stearic acid, C18:0	1.15 ± 0.88 ^{a,AB}	1.43 ± 1.80 ^{a,A}	2.62 ± 0.17 ^{a,A}	0.98 ± 0.23 ^{a,B}	3.14 ± 0.92 ^{a}
	Arachidonic acid, C20:4	0.41 ± 0.20 ^{a}	0.25 ± 0.00 ^{a,A}	0.35 ± 0.02 ^{a,A}	0.34 ± 0.00 ^{a,A}	nd
	Total	35.0	16.2	22.8	12.7	29.7
	AI	0.00	0.02	0.00	0.02	0.01
	TI	0.07	0.18	0.26	0.22	0.25
3rd	Palmitic acid, C16:0	0.33 ± 0.05 ^{a,A}	0.31 ± 0.04 ^{a,A}	0.68 ± 0.36 ^{a,A}	0.42 ± 0.14 ^{a,A}	na
	Oleic acid, C18:1	18.56 ± 5.56 ^{ab,B}	2.08 ± 0.73 ^{a,A}	5.65 ± 1.75 ^{a,A}	19.20 ± 9.25 ^{ab,A}	na
	Linoleic acid, C18:2	8.55 ± 2.73 ^{a,B}	0.44 ± 0.10 ^{a,A}	1.23 ± 0.31 ^{a,B}	6.76 ± 10.14 ^{a,A}	na
	Stearic acid, C18:0	2.96 ± 0.94 ^{a,B}	0.62 ± 0.00 ^{a,A}	0.57 ± 0.27 ^{a,A}	1.54 ± 0.64 ^{a,B}	na
	Arachidonic acid, C20:4	Nd	nd	nd	nd	na
	Total	30.4	3.4	8.1	27.9	na
	AI	0.01	0.12	0.09	0.02	na
	TI	0.24	0.74	0.35	0.15	na

Values represented as mean ± standard deviation obtained from three tissues (pulp, peel, and seed) in different years and maturation stages (1st, 2nd and 3rd cycle); Values with different letter ($^{a\text{-}d}$) along the row indicates significant statistical differences between varieties (Tukey HSD, $p \leq 0.05$). Different capital letter ($^{A\text{-}B}$) along the column indicates significant statistical differences in same variety and fatty acid, in the distinct harvest cycles (years). AI- Atherogenicity Index; TI- Thrombogenicity Index.; na- not analysed; nd- not detected.

fatty acids. DPPH exhibited positive correlations with arachidonic acid ($r = 0.805$), while displaying negative correlations with lipids ($r = -0.894$), and linoleic acid (-0.663), although not statistically significant ($p > 0.05$). Meanwhile, DPPH shown a positive and significant correlation with TPC ($r = 0.999$, $p < 0.05$). The TPC followed the same pattern of DPPH with the same positive and negative correlations, with arachidonic acid ($r = 0.773$), lipids ($r = -0.917$) and linoleic acid ($r = -0.623$). Oleic acid showed positive correlations with total fatty acids ($r = 0.987$) and linoleic acid ($r = 0.533$), and negative correlation with lipids ($r = -0.680$), while linoleic acid had positive relations with total fatty acids ($r = 0.665$) but negative correlations with arachidonic acid ($r = -0.978$). Arachidonic acid displayed positive correlations with DPPH ($r = 0.805$), contrasting with its negative relationship with lipids ($r = -0.455$), total fatty acids ($r = -0.493$). The consistent correlation between arachidonic fatty acid (ω −6), DPPH and TPC suggests a notable pattern in avocado samples, even if no statistical significance were detected.

The observed variability in fatty acid profiles (Fig. 1), spanning the pulp, peel, and seed across different cycles, might be attributed to geographical variations and climate conditions, since the RCF, RCG, and CB varieties were originated from the eastern part of the island, while the QG and *Hass* varieties hailed from the southern region. Different regions experience unique weather patterns each year, and even within the same region, there can be annual variations in climate. These changes impact edaphic conditions (Ferreyra et al., 2016). This theory aligns with findings by Ferreyra et al. (2016), Ranalli et al. (1999), and Ratovohery et al. (1988). They concluded that fatty acid composition is influenced by geography and climate conditions, notably pointing out that colder climates tend to produce higher quantities of oleic acid, the primary fatty acid in avocado fruit. Our results share the same pattern, with the eastern varieties grown in a colder climate than the south ones, having significantly more oleic acid in the pulp.

A key highlight from our research is the detection of arachidonic acid. This fatty acid is indispensable in our diet since animals, including humans, cannot synthesize it. In the realm of agriculture, arachidonic acid in plants triggers defense reactions against phytopathogens, leading to the accumulation of secondary metabolites like polyphenols (Dedyukhina et al., 2014; Wan et al., 2022). It is noteworthy to mention that literature suggests arachidonic acid is seldom found in higher plants, as is the case here (Wan et al., 2022). This discovery undergoes the unique quality of regional avocados.

Within the fatty acids spectrum, polyunsaturated fatty acids (PUFAs) hold significance as they are vital for human growth and overall health. They play a crucial role in determining indices like the atherogenicity and thrombogenicity indexes effects of fatty acids on cardiovascular disease, both of which offer insights into a plant's nutritional value (Ulbricht & Southgate, 1991). The atherogenicity index (AI) evaluates the ratio between specific saturated fatty acids (namely C12:0, C14:0, and C16:0) and unsaturated fatty acids. This index provides insights into the propensity for lipids to adhere cells within the circulatory and immune systems. On the other hand, the thrombogenicity index (TI) calculates the relationship between aforementioned saturated fatty acids

Table 6
Pearson correlation between antioxidants, lipids, total and unsaturated fatty acids.

	DPPH	TPC	Lipids	Total Fatty acids	Oleic acid	Linoleic acid	Arachidonic acid
DPPH	1						
TPC	0.999*	1					
Lipids	−0.894	−0.917	1				
Total Fatty acids	0.119	0.171	−0.551	1			
Oleic acid	0.280	0.330	−0.680	0.987	1		
Linoleic acid	−0.663	−0.623	0.258	0.665	0.533	1	
Arachidonic acid	0.805	0.773	−0.455	−0.493	−0.344	−0.978	1

Values above 0.5 indicates strong correlation, and different signal indicates de orientation of correlation:
(+) indicates positive correlation.
(-) indicates negative correlation.
(*) indicates significant statistical differences ($p \leq 0.05$).

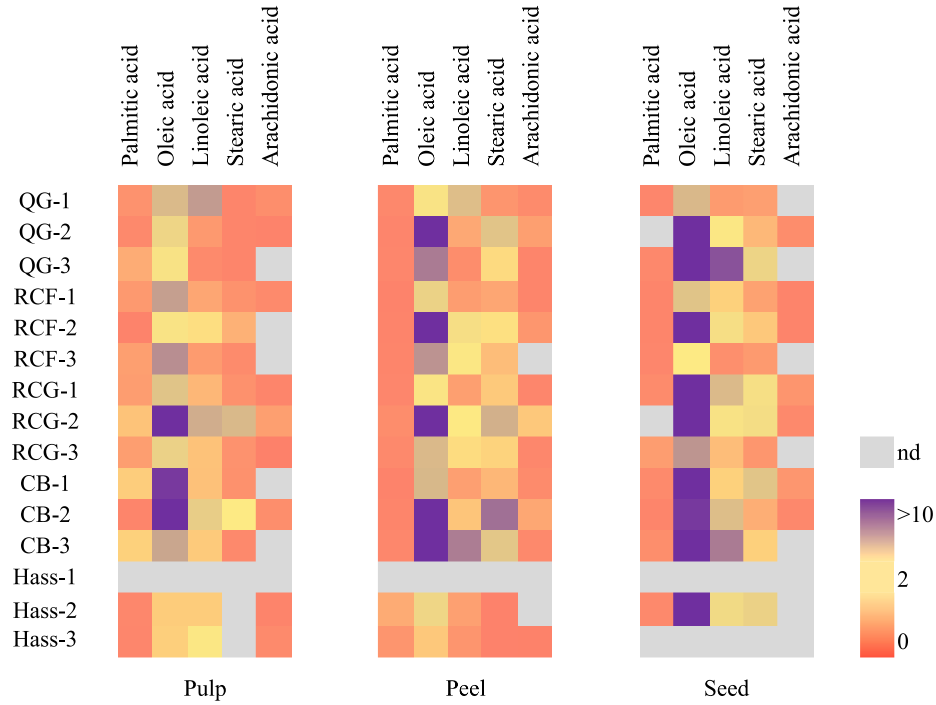

Fig. 1. Variability in the fatty acid profile of the five studied avocado varieties. Values represent the concentration (mg/g) along the harvest cycles (1, 2, 3) in all fruit tissues. QG - *Quebrada Grande*, RCF - *Roxa Casca Fina*, RCG - *Roxa Casca Grossa*, CB - *Cabaça*, Hass - *Hass*, nd - not detected. Fig. 1 – Concentration of fatty acids in mg/g.

(which are pro-thrombogenic) and anti-thrombogenic fatty acids. The latter includes monounsaturated fatty (MUFAs) and polyunsaturated (PUFAs) acids (encompassing both ω-3 and ω-6). This index offers a gauge on the predisposition to form blood clots within the vessels (Chen & Liu, 2020). Both AI and TI indices, when lower, indicate better cardiovascular health. Our study mirrors this, showing values comparable to traditional nuts, that contains high levels of unsaturated fatty acids, which positively influences both indices. The low levels in indices of atherogenicity and thrombogenicity of nuts oils are associated with beneficial health effects, just like our avocados varieties, as they mirror nuts in these indices. Specifically, traditional nuts have low AI indices ranging from 0.07 to 0.14 and TI values between 0.16 and 0.35, as highlighted by Khalili Tilami & Kouřimská (2022).

4. Conclusions

In this study, we analyzed four regional avocado varieties alongside one commercial variety, specifically focusing on antioxidant properties (including polyphenols, DPPH assay, and lipid analysis) and the fatty acid composition of total lipids from avocados cultivated and harvested in Madeira Island. Our objective was to value the avocado fruit and its by-products, understand differences across regional varieties in terms of tissues and harvest cycles, and compare them to the commercial variety.

Our analysis covered various parameters. For antioxidant composition, it was evident that our results slightly surpassed those typically reported in the literature, potentially due to Madeira Island's Mediterranean climate. We identified five major fatty acids present in all varieties. Our findings revealed noticeable differences in these fatty acids across harvest cycles, with occasional distinctions between regional and commercial varieties, in which in general, the regional varieties showed the best quantities of fatty acids when compared with the commercial variety *Hass*, being this a great indicator of the quality of regionals varieties. Furthermore, it acts as an enhancer for the improvement and utilization of these cultivars compared to commercial varieties.

Notably, tree maturation (observed in the third harvest cycle) seemed to enhance the concentration of certain fatty acids, being the best/major quantities of fatty acids registered in regionals varieties.

The quality of avocado by-products showcased in this study highlights in their remarkable antioxidant content, particularly in the peel and seeds, which exhibit superior antioxidant properties. Notably, the peel ranks as the tissue with the highest antioxidant quality. Additionally, it's worth noting that these by-products hold potential applications across various sectors, including the food industry, where they can be utilized to produce natural dyes and emulsifiers.

This study revealed that regional varieties could outperform the commercial ones on some parameters, and its cultivation is imperative for ongoing exploration and appreciation, as well as for utilization, anticipating a potential increase in the consumption of these varieties. However, while these promising results shed light on the potential of avocados from Madeira Island, it's imperative to continue and further delve into this research. Given that this is the pioneering study on these specific varieties, and considering Madeira Island's suitability for avocado cultivation, there's much more to uncover in this domain.

CRediT authorship contribution statement

David Gonçalves: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Carla S.S. Gouveia:** Writing – review & editing, Supervision, Conceptualization. **Maria J. Ferreira:** Writing – review & editing, Methodology. **José F.T. Ganança:** . **Diana C.G. Pinto:** Writing – review & editing, Supervision, Resources. **Miguel A.A. Pinheiro de Carvalho:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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