Comparative Analysis of Biochemical and Nutritional Composition in Regional and Commercial Varieties of Avocado Fruit (Persea americana Mill.)

MASTER DISSERTATION

João David Henriques Gonçalves

MASTER IN APPLIED BIOCHEMISTRY

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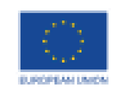

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Abstract

Avocado (*Persea americana* Mill.) is a fruit native to Central America and lately figures as one of the most important and popular tropical fruits in the world due to its nutritional, biochemical and phytochemical characteristics.

In order to evaluate the nutritional, antioxidant and fatty acid composition of four regional varieties and compare them to a commercial one, flours were obtained across different tissues (pulp and by-products) and harvest cycles (different years and on-tree maturation stages).

The nutritional evaluation was carried out using seven parameters covered by crude protein, crude fiber, ash, soluble sugars, starch, lipids, and dry biomass. Was also performed physico-chemical (pH, TA, and color) and pigments (chlorophylls and carotenoids) parameters, and sensory analysis. Among the variability founded in all varieties, in these nutritional parameters, it was revealed the close association of the pulp with the lipid's parameter, while the peel and seed associated to both crude fiber and starch, respectively. In general terms, the on-tree maturation (third harvest cycle) improved the quality of the analyzed parameters.

In antioxidant analysis (TFC, DPPH, and lipids) the by-product exhibited more antioxidant capacity than the pulp, with "Roxa de Casca Fina" (RCF) surpassing the other regionals and commercial varieties in this analysis, especially in lipid content. The fatty acid profile was obtained by GC-MS, which was observed that oleic acid stands out as the major fatty acid founded in all samples, with regional avocados containing arachidonic acid which is an uncommon occurrence among higher plants.

The variability found in these parameters were influenced by the timing of harvest but was notable the major quality of regional avocados in various parameters against the commercial one. This work outcomes highlight the promising potential of avocados from Madeira Island.

Keywords: Avocado; Tropical Fruits; Phytochemical; Antioxidants; Fatty Acids; GC–MS

Resumo

O abacate (*Persea americana* Mill.) é um fruto oriundo da América Central e ultimamente tem se destacado como um dos frutos tropicais mais importantes e populares do mundo, devido as suas caraterísticas nutricionais, bioquímicas e fitoquímicas.

Com o intuito de avaliar a composição nutricional, antioxidante e realizar um perfil de ácidos gordos de quatro variedades regionais e compará-los com uma variedade comercial, foram obtidas farinhas de diferentes tecidos (polpa e subprodutos) e ciclos de colheita (distintos anos e estados de maturação na árvore).

A avaliação nutricional foi levada a cabo pelo estudo de sete parâmetros compreendidos por proteína bruta, fibra bruta, cinzas, açucares solúveis, amido, lípidos e biomassa seca. Também foram realizados parâmetros físico-químicos (pH, TA e cor), pigmentos (clorofilas e carotenoides) bem como uma análise sensorial. Além da variabilidade encontrada em todas as variedades, nestes parâmetros nutricionais, foi revelado a forte associação da polpa ao parâmetro dos lípidos, enquanto a casca e a semente associaram-se a fibra bruta e o amido, respetivamente. De uma forma geral, a maturação na árvore (terceiro ciclo de colheita) aumentou a qualidade dos parâmetros analisados.

Na análise antioxidante (TFC, DPPH e lípidos) os subprodutos exibiram uma maior capacidade antioxidante do que a polpa, com a variedade "Roxa de Casca Fina" a ultrapassar todas as outras variedades regionais e comercial, nesta análise, especialmente no conteúdo lipídico. O perfil de ácidos gordos foi obtido por GC-MS, onde foi observado que o ácido oleico se destaca como o ácido gordo em maior quantidade em todas as amostras, com as variedades regionais a exibirem o ácido araquidónico que não é comum neste tipo de plantas.

A variabilidade encontrada nestes parâmetros foi influenciada pelo tempo de colheita, mas é notável a maior qualidade dos abacates regionais em vários parâmetros em relação à variedade comercial. As descobertas deste trabalho enaltecem o potencial promissor dos abacates da ilha da Madeira.

Palavras-chave: Abacate; Frutos Tropicais; Fitoquímicos; Antioxidantes; Ácidos Gordos; GC–MS

Declaration of authorship

Plagiarism is the deliberate use of ideas, phrases, thoughts, opinions, results and conclusions of other people, without properly recognizing their source. This is a form of cheating and an unacceptable tactic, that represents a serious offense to academic ethics which could trigger negative repercussions academically as well as criminally.

Accordingly, I hereby declare that this thesis and the work presented in it is entirely my own, it is original, and all direct or indirect sources used are acknowledged as references.

Funchal, 14th of February 2024

David Gonçalves

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List of abbreviations

AA	Arachidonic acid
CB	Cabaça
DW	Dry weight
Eq	Equation
FAME	Fatty Acid Methyl Esthers
FAO	Food and agriculture organization
Fig	Figure
FW	Fresh weight
GC-MS	Gas chromatography-mass spectrometry
HASS	Hass
HPLC	High performed liquid chromatography
LA	Linoleic acid
LOD	Limit of detection
LOQ	Limit of quantification
OA	Oleic acid
PA	Palmitic acid
QG	Quebrada Grande
RCF	Roxa de Casca Fina
RCG	Roxa de Casca Grossa
ROS	Reactive Oxygen Species
INE	Instituto Nacional de Estatística
SA	Stearic acid
MBTH	3-methyl-2-benzo thiazoline hydrochloride method
DNS	Dinitrosalicylic acid method
MPOD	Macular pigment optical density
LDL	Low Density Lipoprotein
TA	Titratable acidity
AI	Atherogenicity index
TI	Thrombogenicity index
PCA	Partial Component Analysis
DPPH	2,2-dephenyl-1-picrylhydrazyl
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
TPC	Total Phenolic Content
CVD	Cardiovascular disease
HDL	High Density Lipoprotein

Planet earth is an ecosystem that harbors several sub-ecosystems which, in turn, are the forerunners of different communities. Its sustainability is a much talked about concept these days, as it tries to establish a relationship between the real needs of societies alongside their own desires and urges. Similar to our personal growth, adaptation, and innovation, achieving true sustainability requires reflection on the past, acknowledging and rectifying mistakes [1].

The increase in population and respective food, will necessarily raise food waste, which constitutes a high threat to human well-being, sustainable food, stable food prices, as well as sustainability in general [2]. The world population, by 2050, will be around 10 billion people, and Food and Agricultural Organization (FAO) estimate that food demand will be higher and at least a third of all food goes to waste, which logically will implicate changes in planet ecosystems [3].

Engaging in agriculture, especially in its industrial form, involves the utilization of natural resources, leading to inevitable disruptions in the proper functioning of the natural environment [4]. The growth of sustainability in the agriculture sector is essential since the recent demand for natural and biological products are only achieved with a sustainable agriculture system and, according to FAO, currently 70% of global fresh water is consumed alone by agriculture, which is excessive for a single activity [4].

The analysis and evaluation of the sustainability of plant-based choices become particularly complex, when considering innovative products that are not yet familiar to consumers, which to fight this, in recent years, new plant-based products called as "superfoods" are becoming more appealing to the consumer [5]. Knowing that there is no valid scientific definition for the term "superfood", it is undeniable that there are certain foods that are healthier than others, and this term is informally used to characterize foods with high levels of vitamins, minerals, antioxidants, and phytochemicals in plants that have a positive impact on health and disease prevention [6].

The avocado (*Persea americana* Mill.) belongs to the *Persea* genus and finds utility in various domains, ranging from health and culinary applications, with its most significant

impact lying in the field of medicine [7]. Avocado boasts numerous medicinal benefits attributable to its bioactive constituents, encompassing anti-aging properties, effectiveness in managing heart conditions with hypertensive effects, as well as anti-cancer, anti-inflammatory [8], and anti-microbial activities [9]. These attributes render the fruit highly sought after, not only for consumption, but also for its oil that shares these bioactive compounds and, consequently, avocado and its derivatives remain subjects of ongoing global research endeavors [10].

For the reasons mentioned above, the avocado has become a health promoting agent, becoming one of the most economically important tropical fruits in the world [11]. In Portugal, the production of avocado occurs essentially in Algarve and Madeira Island due to its Mediterranean temperate climate [12].

Therefore, the objectives of this work were to analyze four Madeira Island regional varieties and one commercial, cultivated in Madeira Island, at (i) the nutritional, proximate, and phytochemical level, using all parts of the fruit, (ii) verify the presence or absence of alteration in these same parameters, studied over three harvest cycles (different years and stages of maturation), (iii) as well as make a comparison between regionals and commercial varieties, and (iv) highlight the role of by-products (peel and seeds) and the importance of their use in different areas.

1.1. Avocado

Avocado (*Persea americana* Mill.) is a fruit that belongs to the Lauraceae in the order Laurales, with 50 genera and 2,500 species [13], also it is native to the tropical and subtropical region of Central America, South America, and Mexico, and has expanded to all tropical regions in the world [11,14]. The avocado is among the most economically important of the various tropical and subtropical fruit crops, with its production and consumption increasing systematically over the last 150 years [11].

The avocado fruit (Fig. 1), that resemblance a pear, consists of four main parts: the pericarp, that is composed by three majors parts: the exocarp (11 – 15%), the mesocarp (65 – 73%), and the endocarp, which are usually designated as peel, pulp, and seed husk, respectively, and the seed (16 – 20%) [15]. Typically, when discussing the endocarp, we are referring to the seed, which incorporates the seed husk.

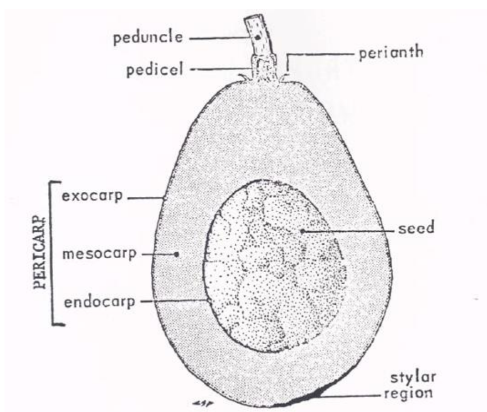


Fig. 1 Avocado morphology. From Storey et al. [15].

Avocado is rich in proteins, fibers, minerals, vitamins, and numerous bioactive phytonutrients that play a significant role in promoting health [16]. Avocados are mainly known for their high lipid content, which these fats and oils are primarily composed of monounsaturated fatty acids, that contribute to their positive impact on human health [10,16]. Avocado stands out not only due to its distinctive composition, but also because of inherent traits common to all avocado trees. These trees exhibit a remarkable flowering period that can last up to three months, resulting in the simultaneous presence of fruits at different stages of development on the same tree. Furthermore, avocados can remain on the tree for as long as twelve months, even though they do not achieve physiological maturity while still attached [17]. The relevance of the fruit is evident in the vast literature publish in last years. The Fig. 2 shows the number of scientific publications related to avocado fruit in the last twenty years, in which, the collected data is based on publications retrieved from PubMed, and Web of Science databases using "avocado" and "avocado oil" as search keywords. Studies related to the avocado fruit have been growing vertiginously in the last ten years.

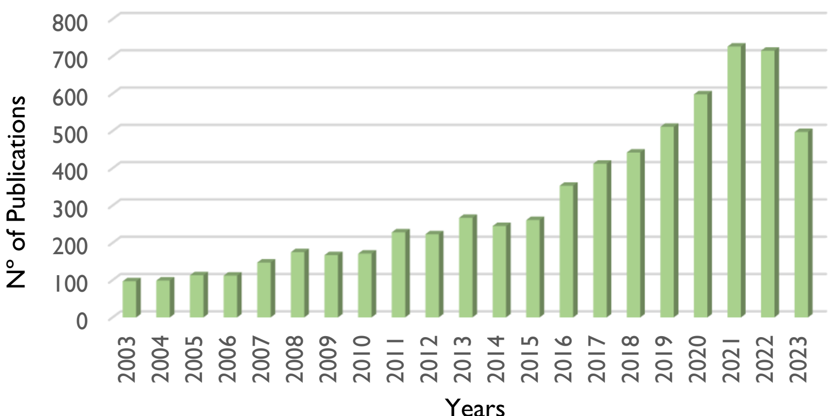


Fig. 2 Publication related to avocado in the last twenty years. Data retrieved from PubMed, and Web of Science databases using "avocado" and "avocado oil" as search keywords.

There is no doubt that avocados are associated to many health benefit, however the concerns about avocado and its environmental sustainability have recently grown with the increased popularity and with its production [5]. Avocados are consumed globally, but generally cultivated in a restrict group of regions of the world, concentrating the water impact and its consequences in avocado production on the planet [3].

The main concern related to avocado waste is the fact that the material (by-products) are discarded in the environment, resulting in ecological problems, like greenhouse gases emissions and soil and water pollution [18]. Globally, the avocado waste, related to fruit discard or by-product waste, is estimated at 40% of the total production [19]. Luckily, there are new alternatives and use of avocado waste for green activities, such as the biorefinery for the production of biodiesel, biogas, bioethanol, biodegradable plastic, and natural fertilizers [19].

It is essential not only to focus on and address the waste generated by avocados, but also to consider the conditions required for their production and their environmental impacts [5]. Avocado is a crop widely dependent on water availability, with its edible part composed by 72% of water, and being a water sensitive cultivar, the lack of water generates losses in the fruit production [18]. Consequently, what is occurring is that these regions are already falling within areas experiencing water shortages, specifically in America (Mexico, Chile) and Europe (Spain) [5].

In general, delving into the structure and characteristics of avocado waste holds significant promise for sustainable waste management, extending beyond avocados to encompass other plant residues as well [19].

1.2. Origin and history

When talking about the origin and history of the avocado fruit, we can note that countries located in the Mesoamerica, namely Guatemala and Mexico, are considered the center of origin of the avocado fruit [20], and the ancient Maya civilization, or its predecessors that lived in this region, probably domesticated the avocado [21].

The avocado (*Persea americana* Mill.) is distinguished and identified following their genetic and morphological differences and has three well demarcated varieties known as *americana*, *guatemalensis*, and *drymifolia* (Fig. 3), which they for long time have been known as West Indian, Guatemalan, and Mexican, respectively [22]. The first variety is a tropical lowland (≤1000 m altitude) cultivated in tropical and subtropical regions, where can be observed be detected numerous cultivars and ecotypes, while the other two races are

tropical highlands (1000 – 3000 m altitude), widely grown in warm (Guatemalan) to cool (Mexican) subtropical and Mediterranean climates [23].

Fig. 3 Varieties of avocado: *Persea americana* var. americana (left); *Persea americana* var. guatemalensis (mid); *Persea americana* var. drymofolia (right). Image credit: http://avocadosource.com/

Historical records indicate that avocados have a long history, dating back approximately 10,000 years ago [13]. The oldest known avocado fossil, estimated to be around 8,000 years old, was discovered in Mexico [24]. Its name, "Ahuacatl," originates from the Nahuatl language, which as various translations, including "testicle," owing to the fruit's distinctive shape and resemblance, and also alligator pear for shape and peel resemblance [25]. Moreover, it has spread across the globe, acquiring various names such as "avocado" in English, "avocat" in French, "aguacate" in Spanish, and "abacate" in Portuguese [26].

In the Mayan era, the months of the civil calendar were attribute to seasonal events or important agricultural events, which reveals the high impact on the lives of the people when they named their 14th month after the avocado fruit [27].

This nutritionally rich fruit has garnered significant recognition and holds substantial commercial value worldwide [28].

In Europe, due to its Mediterranean and temperate climates, Spain, Greece, Italy, and Portugal are the countries where avocados are most widely grown [12].

1.3. Avocado in Portugal

As mentioned before, in Europe, Portugal is one of the countries producing avocados, namely in the Algarve and in Madeira Island due to favorable edaphic and climate conditions [12]. The *Hass* and *Fuerte* varieties take center stage as the most extensively cultivated avocados worldwide (including Portugal), exerting dominance in the international market [29]. Following the data released in 2022 from Portuguese statistical institute about agriculture production, in the latest three years (2020, 2021, and 2022), avocado has increased its production and cultivated area [30]. In Fig. 4, the area for avocado cultivation, in Portugal has grown from 2,341 ha in 2020, to 3,194 ha in 2022, and the

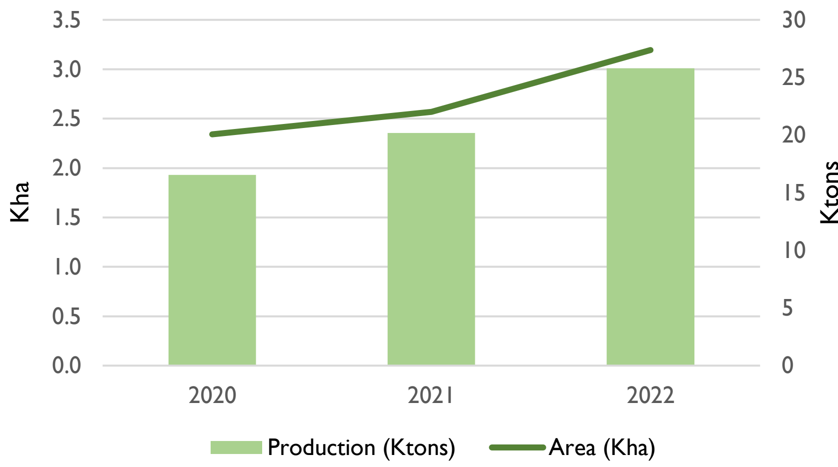


Fig. 4 Evaluation of area and production of avocado in Portugal. Data from INE (2023) [30].

production as also being increased in these years, growing from 16,555 tons in 2020, to 25,791 tons in 2022.

In Madeira Island, the introduction of the avocado took place in the 19th century, brought from Brazil, where it was known for the shade it provided to the detriment of the fruit, as described by the agronomist Leandro Aguiar Camara in his report "Fruticultura subtropical na ilha da madeira" in 1955, where he quotes the well-known researcher Alberto Sarmento [12]. The area showcases significant agricultural diversity in this crop, thanks to the introduction and widespread cultivation of genetic material since the mid-nineteenth century. This genetic diversity encompasses resources from both the West Indian (*Persea americana* Miller var. americana) and Mexican (*Persea americana* Miller var. drymifolia) gene pools [31]. Fig. 5 shows that cultivation area and production of avocado, in Madeira, also increased growing from 50 to 51 ha, and from 462 to 498 tons, in 2020 to 2022, respectively.

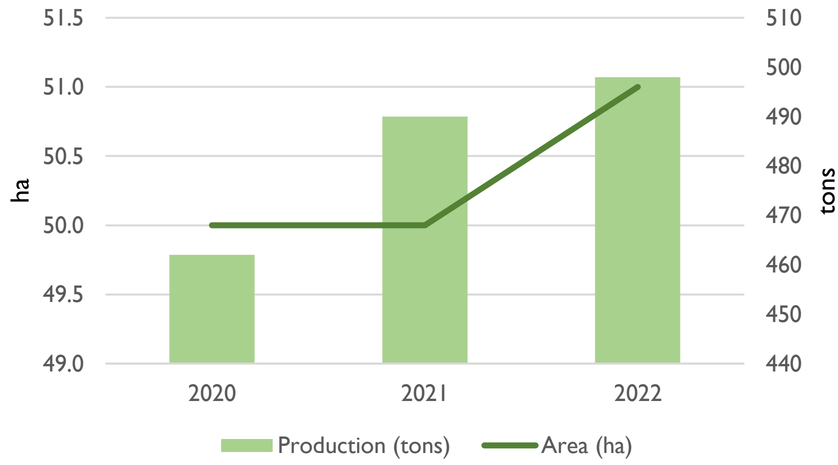


Fig. 5 Evaluation of area and production of avocado in Madeira Island. Data from INE [30].

In Portugal, the "Bacon" and "Pinkerton" varieties are also cultivated, as they adapt well to Portuguese edaphic conditions. Harvesting season consider the geographic location, weather, altitude, and variety, but, normally, is from June to October [12].

1.4. Free radicals

In living organisms, there are biochemical and physiological processes that can form damaging substances called "free radicals", that are responsible for oxidative damage of lipids, proteins, and DNA, which could lead to chronical diseases like cancer, diabetes, aging, and degenerative diseases [32]. Reactive oxygen species (ROS), which are free radicals characterized by exceedingly unstable species possessing an unpaired number of electrons in their outer orbit, and have a propensity to engage oxidative or reductive interactions with healthy cells, resulting in the degradation of cellular structure and function [33]. This process takes place in the cytoplasm, mitochondria and/or cell membranes, resulting in a series of reactions as the attacked molecule loses its electron and becomes a free radical that damages other living cells. As told before, oxidative stress is the major prejudicial effect that free radical has and it occurs due to the imbalance existing between the formation of reactive oxygen species and the cells ability to eliminate them [34].

Since the mitochondria is the main consumer of oxygen available in the body, around 90%, naturally, this organelle serves as the primary location for the free radical's generation, particularly ROS. In plants and animals, during the cellular respiration, only 98% of the O_2 consumed by mitochondria is reduced in water, and 2% is partially reduced to toxic O_2 which, during the sequentially transference of four electrons to the O_2 molecule, catalyzed by the cytochrome oxidase, can originate anion superoxide ($O2•^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•) additional to water (Fig. 6) [35].

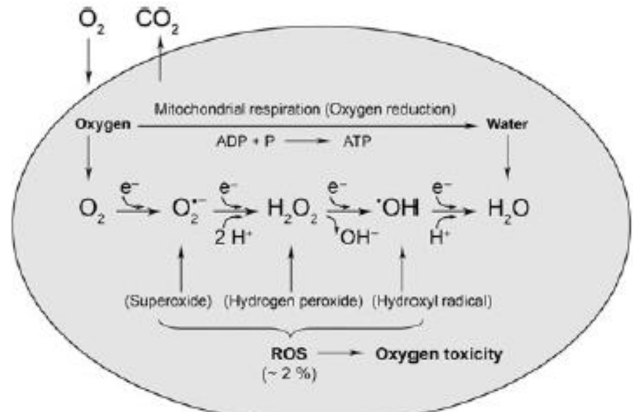


Fig. 6 Formation of reactive oxygen species (ROS) in mitochondrial respiration. From Kutschera and Niklas. [35]

Another process where the release of free radicals occurs is phagocytosis, which during the destruction of cells infected by bacteria or viruses, provide the release of oxidizing compounds [36]. The endogenous pathways are not the only source of free radicals, as there is a set of exogenous processes that are responsible for the appearance of these species, standing out in industrialized countries, radiation, smoke, and polluting agents [37]. Avocados are closely associated as a potential source of antioxidant to combat and fight oxidative stress, due to its nutritional and phytochemical attributes, notably the presence of beneficial fatty acids essential for every cell in the human body, a compelling illustration is the fact that approximately 60% of the brain's composition is comprised of fat and, additionally, there is documented evidence of the utilization of avocados in addressing various conditions, including hypertension, diarrhea, and diabetes [38].

1.5. Bioactive compounds

Bioactive compounds are subject to several factors that influence their composition in certain products, especially fruits, where edaphoclimatic conditions, state of maturation and harvest times play an important role [39]. It is known that the consumption of fruits by the consumer, provides him with a greater ability to combat certain types of abnormalities in the human body, e.g. diseases, even severe diseases such as chronic diseases and certain types of cancer, all due to the presence of bioactive antioxidant compounds [40]. Naturally, these bioactive compounds, specifically phytochemicals and secondary metabolites, confer to fruits its antioxidant capacity and being avocado a fruit with higher levels of bioactive compounds and consequently high antioxidant capacity, it is common for this fruit to become a subject of increasing attention and hope is deposited in trying to help resolve any of these “problems” [41].

Antioxidant molecules can be defined as substances that, when are present in a substrate, can delay or even inhibit its oxidation by free radicals acting as acceptors or donators of electrons to eliminate the unpaired connection of the free radical. These molecules have the potential to engage directly with the reactive radical molecules, neutralizing them. In the process, they transform into new, less reactive molecules that pose reduced danger to the human organism [42].

1.5.1. Carbohydrates

Carbohydrates are organic compounds with a chemical structure of [$C_nH_{2n}O_n$] that are widely spread in nature. In food domain, these compounds have an important role, as

they are the major energy source of human metabolism, being its recommended intake at 200 g per day [43,44].

These compounds are founded in animal and plant tissues, and many are the functions related to them, like energy reserve (starch, glycogen, and fructans), structural (cellulose, xylene), protection (polysaccharides as elicitors of plant antibiotics) and cellular recognition (glycoproteins and glycolipids) [43].

Carbohydrates are classified according to the molecular size, which is determined by the degree of polymerization, the character of individual monomers and the type of linkage (α or β). Carbohydrates can be divided into three groups: sugars (monosaccharides, disaccharides, and polyols), oligosaccharides, and polysaccharides (starch) [45].

Its known that avocado has very little sugar when compared to other fruit, especially tropical fruits, which vary from around 0.2 g to one-half of avocado [8].

The dominated soluble sugar is seven-carbon (C7) D-mannoheptulose, and it reduce form polyol, perseitol (Fig. 7), whereas six-carbon (C6) like glucose, fructose, and sucrose are in lower concentrations. Sugars are associated with some roles in biological process in fruits, like the growth and development, but also is associated to fruit ripening by acting like a respiratory substrate [46]. It is also thought that this type of seven-carbon sugar is a unique phytochemical characteristic of the avocado fruit [8].

In prior research, it was noted that D-mannoheptulose and perseitol are found in higher concentrations in unripen avocado fruits, with their levels decreasing as the ripening process advances, acting as inhibitors of the ripening process, which has positive implications for the commercial transport and storage of the fruits [47]. The presence of these seven-carbon sugars in substantial quantities within avocado trees is of biochemical interest, as they constitute the primary forms of nonstructural carbohydrates in the tree, but despite their importance and intriguing nature, there remains limited knowledge regarding the metabolism and biochemical synthesis responsible for producing these seven-carbon sugars [48]. Another significant role played by C7 sugars in avocados involves carbon allocation processes within the tree, particularly their function in storage reserves and primary CO_2 fixation as phloem mobile products [49].

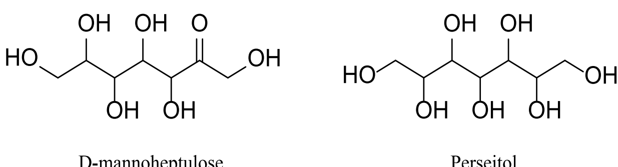


Fig. 7 Sugars C7 found in avocado fruit. Figure from author.

In general terms, the base of soluble carbohydrates is the monosaccharide glucose, which, when combined, forms total soluble sugars (TSS). Glucose can engage with a lot of compounds, and the determination of the TSS is a very common and simple analytical procedure. Sugars can be analyzed by different methods, like chromatographic and colorimetric, being the first one the most used, due to its high sensitivity and the ability to quantitate sugar individually, conversely its complicated, expensive and time consuming [50]. Many are the colorimetric methods for the detection of carbohydrates, like phenol-sulphuric acid [51], orcinol-sulphuric acid method [52], 3-methyl-2-benzo thiazoline hydrazine hydrochloride (MBTH) method, dinitrosalicylic acid (DNS) method, Somogyi and Nelson's [53], but, without a question, the anthrone-sulphuric acids is the most common and used technique [54]. The phenol method is indicated for the determination of polysaccharides with different types of sugars in its composition. It is a very sensitive, rapid, simple, inexpensive, and reproducible test, but has the issue that phenol is carcinogenic, and its vapors damaging skin, eyes, and respiratory system, which make this procedure unfeasible for some researchers [55]. The orsinol-sulphuric acid method is indicated for polysaccharides and simple sugars, but is not much used because it's quite complicated, depends of a lot factors, and it's not very sensitive [56,57]. The MBTH method was initial proposed to detect aliphatic aldehydes, but later was redirected to sugar detection and quantification, however this procedure is conditionate due to its low detection limits and danger reagents [53]. Both DNS and Nelson's methods are appropriate to quantify reducing sugars. While DNS is rapid and easy to perform, its major limitations is quantification of individual sugars, which can be challenging, due to the varying intensities of colors given by different reducing sugar (sugars with more linkages are more oxidized), and the used of alarming reagents [53]. Nelson's is time-consuming and require hazardous reagents [53]. The anthrone-sulphuric acid method is the best and the desirable for the determination of carbohydrates composed by glucose [58]. The method is based on furfural derivatives originated from carbohydrates in the existence of concentrated sulphuric acids with anthrone (9,10-dihydro-9-ozoanthracene), originating blue-green complexes [53].

1.5.2. Carotenoids

Carotenoids are one of the most important classes of compounds belonging to natural pigments, which play several roles in the human body due to their wide distribution and structural chemical diversity. Consuming avocados and numerous other fruits and vegetables that are abundant in carotenoids is linked to a decreased likelihood of

developing various diseases, largely due to their antioxidant properties, which are linked to cell protection mechanisms, regulation of cell growth and targeting and in processes related to controlled cell apoptosis [59]. The major carotenoids found in avocado are from a subclass known as xantophylls, more specifically, lutein and zeaxanthin (Fig. 8), which predominate over the other carotenoids subclass, carotenes, and constituting about 90% of total carotenoids [8]. It is notable the high variability of carotenoids in avocado fruit with, for example, the lutein (the highest carotenoid in avocado) content could ranging from 140 to 842 μg/100g [60].

Fig. 8 Two major carotenoids found in avocado. Figure from author.

The carotenoids of the xanthophylls subclass, which predominate over the other carotenes subclass, constitutes an added value for the above fruit but also tempts for those who consume it since it has a lower capacity to suffer pro-oxidation activities and consequently to form or enhance the onset of disease [61]. Some carotenoids, after ingestion in the body, are transformed into vitamin A, which is an important vitamin in terms of visual health as it participates in the reception of light by the retina. The lutein present in large amounts in avocado is very important for many processes related to eye, and cognitive health, because once in the body, it is stored in the eyes and brain, while other carotenoids are stored in the adipose tissue and liver tissue [16]. Notably, lutein is concentrated in the central area of the retina known as the macula, offering protection against harmful blue light and oxidative damage, thus contributing to the prevention of macular degeneration [16]. Within the brain, lutein remains within neural tissue, and the macular pigment optical density (MPOD) serves as a biomarker for brain lutein. This is due to the correlation between macular and brain lutein, indicating that elevated MPOD levels are associated with increased lutein levels in the brain [62].

Avocado show some evidence that with the passing of the harvesting season, and already in a late phase of the same, the levels of carotenoids are higher than those existing in an initial harvesting phase [63].

1.5.3. Vitamins

Nowadays, many groups of compounds are associated with human health benefits, including vitamins, and many are the roles that vitamins have in the human body, since processes related to metabolism and cell regulation, to growth, development, and nutrition [64]. Since vitamins are a heterogeneous group of micronutrients that cannot be synthesized endogenously, this forces us to obtain them, through the food [65]. Similar to other compounds, vitamins are categorized based on their solubility, which can be classified as either fat-soluble vitamins (liposoluble) such as vitamins A, D, E, and K, or water-soluble vitamins (hydrosoluble) like those found in the B-complex and vitamin C [64].

The avocado fruit stands out for having in its composition two of the most important vitamins (Fig. 9), vitamin C (ascorbic acid) and vitamin E (DL-alpha-Tocopherol).

Ascorbic acid

DL-alpha-Tocopherol

Fig. 9 Two major vitamins found in avocado fruit, ascorbic acid (hydrosoluble) and DL-alpha-Tocopherol (liposoluble). Figure from author.

The vitamin C, it's probably the most important and known vitamin, indispensable to normal life as its participated in many important physiological processes [64]. It serves as a potent antioxidant, safeguarding molecules against oxidative harm, contributing to collagen biosynthesis and tyrosine metabolism. These functions are linked to promoting vascular health and stabilizing the arterial system. Additionally, it plays a crucial role in preserving and steadying the protective circulatory system, specifically by retarding LDL cholesterol oxidation, as discussed by Dreher & Davenport[8]. Vitamin E it is known as the most important lipid-soluble antioxidant that participated in biological systems, with the main function protecting polyunsaturated fatty acids in membranes and lipoproteins as chain-breaking antioxidant against the propagation of free-radicals [66]. Furthermore, vitamin E is found in nature as tocopherols and tocotrienols in four different forms (α, β, γ, and δ), being α-tocopherol the most active antioxidant form distributed in plant tissues and it is used in other cell functions, such as gene expression, immune functions, but is also

used in many fields like cosmetic, pharmaceuticals, resins, and especially in food industry, since its antioxidant properties that are a requirement for these industries [65,66].

1.6. Antioxidants compounds

In the realm of fruits, specifically within avocados, numerous factors contribute to the range of antioxidants compounds found in various cultivars. These factors encompass elements like soil and climate conditions (including light, temperature, water, and soil nutrients), maturation, harvesting methods, post-harvest handling, processing techniques, and storage conditions [67].

There is much evidence that the fruit, in general, has properties that provide protection to the human body, namely against pathologies. These characteristics usually being associated with antioxidant properties. Naturally occurring antioxidant bioactive compounds found in diverse fruits, it heightened focus from the scientific community [60].

1.6.1. Phenolic compounds

Incorporating fruits and vegetables into one's diet is linked to favorable health outcomes, largely attributed to the biological actions of phenolic compounds and other bioactive constituents (Fig. 10) [68]. Phenolic compounds, found abundantly in the plant kingdom, typically serve as secondary metabolites offering protection against various stressors, including climate change, pathogens, radiation, and more, other abiotic or biotic stressors [69]. Present in many protective processes, was demonstrated that this class of secondary metabolites are good ROS scavengers [70]. The pursuit of phenolic compounds has intensified among researchers due to the numerous favorable characteristics exhibited by them, which has led to an increased focus on exploring fruits, vegetables, and plants, as well as agricultural and agro-industrial residues as potential sources of these compounds [71].

Phenolic compounds are part of a large and heterogeneous metabolites group, with more than 8000 compounds being differentiated and classified according to their molecular structure. The base molecular structure has the presence of aromatic ring (one or more) and hydroxyl groups (at least one) [72]. This class of compounds are divided in two major subclasses, being one the phenolic acids that are simple molecule with one hydroxyl group (phenol group), and the flavonoids which are complex molecules with two or more phenol groups [68].

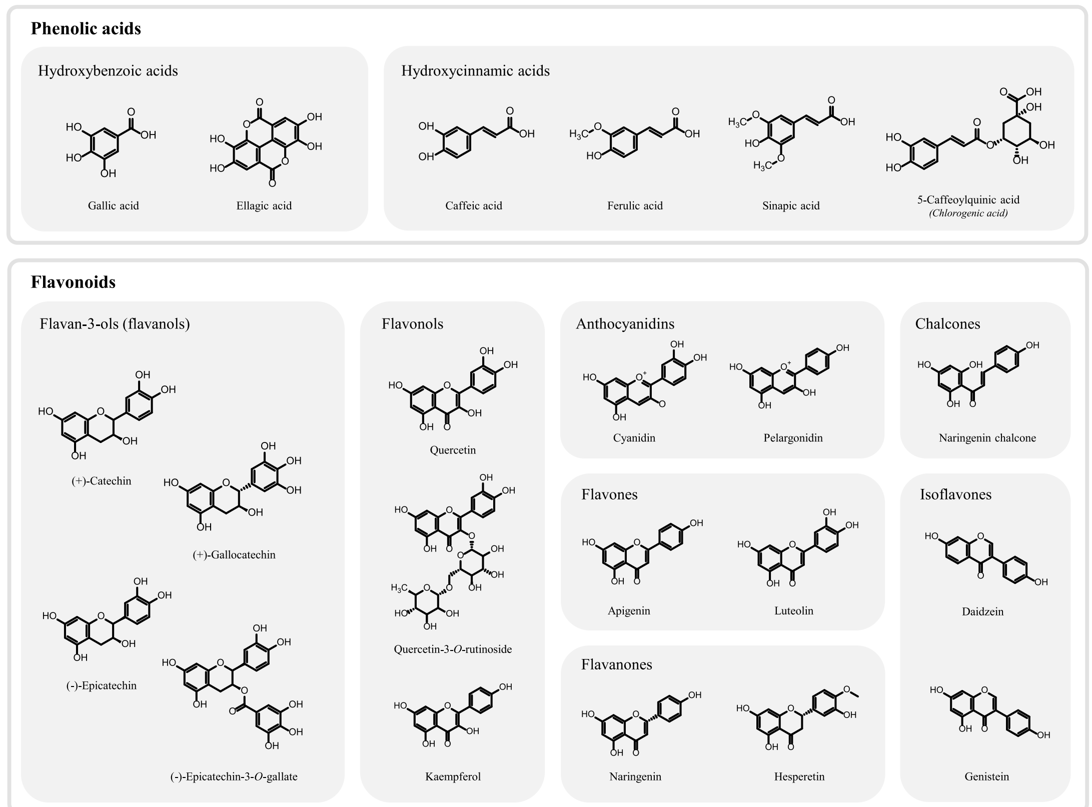


Fig. 10 Most common phenolic acids and flavonoids founded in avocado fruit. Adapted from Ribas-Agusti et al. [72].

Phenolic acids can be divided in two structures, hydroxybenzoic (Fig. 11) and hydroxycinnamic acids (Fig. 12) [72].

R_1, HO, COOH, R_2

$R_1=R_2=H$; p-Hydroxybenzoic
$R_1=OH$, $R_2=H$; Protocatechuic
$R_1=OCH_3$, $R_2=H$; Vanillic
$R_1=R_2=OH$; Gallic
$R_1=R_2=OCH_3$; Syringic

Fig. 11 Structure of hydroxybenzoic acids. Figure from author.

R_1, HO, C=CH–COOH, H, R_2

$R_1=R_2=H$; p-Coumaric
$R_1=OH$, $R_2=H$; Caffeic
$R_1=OCH_3$, $R_2=H$; Ferulic
$R_1=R_2=OCH_3$; Sinapic

Fig. 12 Structure of hydroxycinnamic acids. Figure from author.

These acids derivatives are composed by structures with nine carbon atoms (C6 – C1) and seven carbon atoms (C6 – C3), respectively, and normally they are found in the hydroxylated form [73]. Undoubtedly, among numerous protective and antioxidant

processes, the paramount role lies with ROS scavengers, and their efficacy within these phenolic compounds hinges on the number and location of hydroxyl (-OH) and methoxyl(-OCH_3) groups within the molecules [74]. In the realm of antioxidants, hydroxycinnamic acids exhibit superior antioxidant potential due to the presence of a propenoic group in the side chain instead of a carboxyl group in hydroxybenzoic acids. The double bond exerts a stabilizing influence on the molecule through resonance with the phenoxyl radical, thereby augmenting the antioxidant attributes of the aromatic ring [70,75]. In the food industry, the phenolic acids present in greater quantities are the hydroxycinnamic acids derivatives [75].

Regarding food industry, phenolic compounds have been used as natural antioxidants, natural coloring agents, nutritional additives, and chelating agents [76]. There been many studies regarding the bio-preservative action of these compounds, in the improvement of the shelf-life of perishable products [18]. Since these compounds in plants are very abundant in these compounds, it is not surprising that in approximately 500,000 species of plants, 1-10% are used in food kitchens worldwide [77].

The primary phenolic acids found in avocados include gallic acid from the hydroxybenzoic acid group and caffeic and sinapic acid from the hydroxycinnamic acid group, and the well-established literature on avocados highlights the recognized rich anti-inflammatory and antioxidant properties of this class of compounds [78]. In numerous instances, a clear correlation exists between the quantity of phenolic compounds and their efficacy in combating and reducing factors such as oxidation and inflammation, fundamental for fruit protection. Additionally, it is evident that by-products of the fruit exhibit a higher antioxidant capacity compared to the edible portion [60].

1.6.2. Flavonoids

Flavonoids constitute an extensive group of low-molecular-weight secondary metabolites characterized by two interconnected aromatic rings within a three-carbon chain. They have the potential to cyclize, forming a third ring, while maintaining a fundamental C6-C3-C6 structure. These compounds are synthesized in all parts of the plant and are widely distributed throughout the plant kingdom [79].

Flavonoids serve various functions, with one crucial role being their contribution to the flavor, scent, and pigmentation of fruits, flowers, and seeds. This, in turn, enhances their appeal to insects and birds, ultimately facilitating pollination and seed dispersal, which is a vital process in the growth of these plants [80].

This group can be divided into many classes according to the degree of oxidation of oxygenated heterocycle, flavones, flavonols, anthocyanins, chalcones, flavan-3-ols, etc., and generally occurs in plants by additional hydroxyl, methoxyl, methyl or glycosyl substitution patterns [81].

The primary flavonoids found in avocados are flavonols, anthocyanins, and proanthocyanidins, which are known for their strong antimicrobial and antioxidant properties. These compounds also play a significant role in the inhibition harmful processes within the body [29,39].

1.7. Avocado Oil

Like all fruits and nuts rich in lipids or fats, their respective oils are characterized by a high number of bioactive compounds that play a significant role in maintaining and regulating human health. Avocado is one of the fruits with the highest percentage of oil (considered an oleaginous fruit), with its pulp containing approximately 60% lipids, followed by the peel and seed, which contain 7% and 2%, respectively [82]. Many countries around the world produce avocado oil, but the primary producers are located in Central (Mexico) and South America, South Africa, and New Zealand [83].

The many extraction methods that can be used to recover the avocado oil are, the cold press method, ultrasound-assisted aqueous method, supercritical CO_2, subcritical CO_2, enzymatic and solvent extraction, being the last one, the one with higher extraction yield of fatty acids [10]. Actually, from an industrial point of view, avocado oil has a big advantage over olive oil since it can be obtained by cold extraction method, which is an easy and low-cost technique that preserves all the bioactive compounds in the oil, when other non-cold press methods could change the final physical and chemical composition of the product [83].

Avocado oil is a cholesterol-free oil, rich in monounsaturated fatty acids (mainly oleic acid) and low in saturated fatty acids, which is associated to many health benefits, and contrarily to other oils, avocado oil contains high amounts of sterols and other unsaponifiable components [84]. This composition of sterols, unsaponifiable matter, and fatty acids helps to reduce bad cholesterol and increase good cholesterol, which in itself reduces the likelihood of cardiovascular diseases [85].

1.7.1. Unsaponifiable Components

Avocado oil has a high content of unsaponifiable compounds (4-12%) when compared to most vegetable oils [83]. Unsaponifiable compounds could be defined as

compounds present in both vegetable and animal oils, insoluble in water and not capable of undergoing changes when subjected to saponification reactions, that involves the degradation of lipids [86]. Among the main constituents of this type of compounds in vegetables oils, some stand out like, sterols, aliphatic alcohols, terpenes, hydrocarbons, and tocopherols [87]. From the sterol class, the β-sitosterol is the predominant sterol found in avocado oil [83].

1.7.2. Phytosterols

Phytosterols, also known as plants sterols, are naturally occurring compounds found in the plant world, with their highest concentrations typically found in fatty foods. Structurally are similar to cholesterol but different in side-chain configuration (Fig. 13) and are classified as triterpene compounds, existing in various forms within plants [88]. The most prevalent ones include β-sitosterol, campesterol, and stigmasterol [83].

Fig. 13 Similar structures of most common phytosterols and cholesterol. From Woyengo et al. [89].

These compounds can be defined by three main classes: 4-desmethylsterols, 4-methylsterols, and 4,4'-dimethylsterols (triterpene alcohols), in which the first one, the highest present in avocado, is associated to benefits in health protection, namely in lowering LDL cholesterol (often described as bad cholesterol) offering protection to cardiovascular diseases, anti-cancer, antioxidant and hepatoprotective properties [83,88].

The effectiveness of these components in carrying out their functions hinges on the essential stability of phytosterols, which can be influenced by many factors like their chemical structure, including ring or side chain unsaturation, as well as variables such as time, temperature, and substrate composition [90].

1.7.3. Fatty acids

Fatty acids are lengthy hydrocarbon chains, which can be categorized into four distinct classes: saturated, monounsaturated, polyunsaturated, and trans fats. These fatty acids are prevalent in various food sources, including vegetables, fruits, nuts, as well as animal oils and fats. More than 20 different types of fatty acids can be identified in these foods, and they play a vital role in the human diet, because the human body lacks the biochemical pathways needed for synthesizing these compounds internally [91]. They are engage in numerous biological processes, serving as precursors for various plant metabolites, and additionally, they hold significant importance as components within cellular membranes and storage lipids [92]. Also, these are linked to other health advantages, encompassing cardiovascular benefits assessed through both atherogenicity (examining lipid adherence to cells in the circulatory and immune systems) and thrombogenicity (evaluating the propensity for blood clot formation in vessels) indexes, as well as inflammatory effects [8].

As previously mentioned, these fatty acids can be categorized into four distinct classes, each characterized by variations in their biochemical structure. In the case of saturated fatty acids, they possess carbon-carbon bonds saturated with the maximum possible number of hydrogen atoms. On the other hand, when one or more double bonds (insaturations) appear within the carbon chain, they are classified as unsaturated fatty acids, specifically, they are referred to as monounsaturated fatty acids when there is a single double bond and or as polyunsaturated fatty acids when there are two or more double bonds present [91].

Trans fats represent a specific subtype of unsaturated fatty acids. In trans fats, instead of the typical cis conformation of the double bond, where the two adjacent hydrogens are displayed in the same side of the carbon chain, the hydrogens are arranged in a trans conformation, with them positioned on opposite sides of the carbon chain [91].

In the realm of metabolomics, where the identification of metabolites holds paramount importance, gas chromatography (GC) was the pioneering technique in this field, and despite the emergence of other methodologies like liquid chromatography (LC) and nuclear magnetic resonance (NMR), GC continues to maintain a prominent presence [93]. While gas chromatography-flame ionization detector (GC-FID) continues to be a reliable method for fatty acid analysis, the gas chromatography–mass spectrometry (GC-MS) has gained widespread usage in both quantifying and identifying fatty acids with chain lengths ranging from C8 to C26 [94].

This enduring popularity is attributed to its seamless compatibility with mass spectrometry (MS) through electron ionization (EI) as an ion source, ensuring exceptional reproducibility and robust fragmentation. Moreover, the existence of comprehensive databases facilitates the straightforward detection and identification of metabolites [95]. Certainly, when it comes to the analysis, quantification, and identification of fatty acids, the combination of gas chromatography (GC) with mass spectrometry (MS) stands out as an extensively employed system, which this approach consistently yields results with exceptional sensitivity and precision [96].

In order to proceed to the quantification of fatty acids in oils and fats in GC, a process call derivatization is required, aiming to modify an analyte in order to enhance is detectability in GC [97]. Or in other words, to transform the analytes into more volatile and non-polar derivatives, which can be easily detected, after extracting lipids from the food and before GC analysis [94]. In literature, three majors derivatizations approaches are spoken, alkylation, acylation and silylation [97], and different methods are used from food samples to prepare fatty acid from lipids, such as, acid- or base-catalyzed transmethylation, borontrifluoride (BF3) methylation after hydrolysis, methylation with diazomethane and silylation [98].

Methylation is the predominant fatty acid derivatization technique, resulting in the formation of the commonly employed (FAME). However, the silylation of lipids has garnered considerable research attention, not only for determining the positioning of fatty acids within the glycerol backbone but also for characterizing crude vegetable oils and their associated co-products [99]. Also, silylation is used on specific analytes or directly on complex samples, like plants material [100].

The process of silylation is a chemical reaction in which all reactive atoms of hydrogen, such as OH, COOH, SH, NH, etc., are replaced by a silyl group, frequently with trimethylsilyl (TMS). This method proves its reliability by effectively reducing the polarity of the analytes, enhancing their stability, and ultimately enhancing GC performance as desired. Silylation reactions exhibit several factors that contribute to improved efficiency, such as the silyl donor capabilities of the reagent and the ease of silylation across various functional groups present in the analyte [100]. The silylation process (Fig. 14) happens through nucleophilic attack, where the better the leaving group, the better the silylation [97]. These reagents used in this derivatization method can be in pure state, or in mixtures of two or more reagents, which is preferable as a mixture provides better efficiency in the silylation of specific compounds [100].

$$\text{Sample}-\ddot{\text{O}}(\text{H}): + \text{CH}_3-\text{Si}(\text{CH}_3)_2-\text{X} \longrightarrow [\text{Sample}-\overset{\delta+}{\ddot{\text{O}}}(\text{H})-\text{Si}(\text{CH}_3)_2-\overset{\delta-}{\text{X}}] \longrightarrow \text{Sample}-\text{O}-\text{Si}(\text{CH}_3)_2-\text{CH}_3 + \text{HX}$$

Fig. 14 Silylation reaction. From Orata [97].

1.7.3.1. Saturated fatty acids

Many studies have shown that the consumption of foods rich in saturated fatty acids (SFAs) is associated with an increased diseases risk, including increased blood cholesterol levels, which consequently enhance the severe cardiac risks [101]. The diseases risk increase is not related to all fatty acids, as it has already been shown by some studies that short-chain fatty acids (less than 12 carbon atom), and steric fatty acids, have much less tendency to increase LDL-cholesterol values, while long-chain fatty acids (12, 14, and 16 carbon atoms) significantly increase the cholesterol values [91].

Among the saturated fatty acids posing the greatest risk to both animal and human health, myristic acid stands out as the most harmful, with lauric acid coming next, and palmitic acid ranking lowest in terms of harm [102]. Inside the avocado fruit, saturated fatty acids make up about 14-16% of the total fatty acid composition, with palmitic acid (Fig. 15) being the dominant component [8,47]. Fortunately, it is considered the least harmful among its saturated counterparts [60].

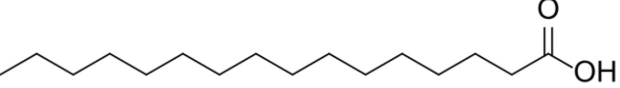


Fig. 15 Saturated fatty acids – Palmitic acid. Figure from author.

1.7.3.2. Monounsaturated fatty acids

Plants produce a high diversity of unsaturated fatty acids that differ in number of C-C bonds, that affect the chain length, and the existence of double bonds. Monounsaturated fatty acids (MUFAs) are among these fatty acids, defined by a unique double bond in its straight-chain (unbranched) [103]. Dietary lipids impact organ metabolism as they undergo assimilation in the gastrointestinal tract and subsequently get transported throughout the entire body [104]. In contrast to saturated fatty acids (SFAs), which are associated with an increased risk of diseases, particularly heart diseases, evidence indicates that monounsaturated fatty acids (MUFAs) not only reduce this risk but also help mitigate various factors that could otherwise raise it [105]. In the majority of the fruits, during maturation time, the content of unsaturated fatty acids (monounsaturated and

polyunsaturated fatty acids) becomes superior [106], even more in MUFAs if the temperature decreases, as it been showed an increase in oleic acid with lower temperatures [47]. The MUFA content in avocado reaches around 71% of total fatty acids, being the most abundant, and the major fatty acid overall in avocado fruit, is oleic acid (18:1ω-9) (Fig. 16) [8].

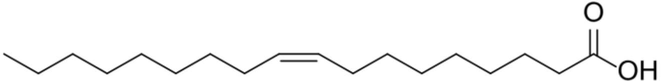


Fig. 16 Monounsaturated fatty acid – Oleic acid. Figure from author.

1.7.3.3. Polyunsaturated fatty acids

Just like MUFAs, polyunsaturated fatty acids (PUFAs) are crucial to the vital function of human activity, as they participate in many syntheses in the body, also are linked to brain development and cognition, and their absence causes damage changes in a wide range of organs like heart, kidneys, and liver [92]. Furthermore, PUFAs, as they are precursors of eicosanoids, that controls inflammatory processes and immune responses.

The major PUFAs are the omega-3 polyunsaturated fatty acid (ω-3 PUFA) and omega-6 polyunsaturated fatty acid (ω-6 PUFA), represented in majority by the essential fatty acids, alpha-linolenic acid (ALA,18:3ω-3), and linoleic acid (LA,18:2ω-6), respectively [107]. These PUFAs linoleic and alpha-linolenic acid could be converted into its homologues C20 eicosanoids, arachidonic acid (20:4ω-6) and eicosapentaenoic acid (EPA) (20:5ω-3) enzymatically, in a process that involve elongations of the acyl chain, and desaturation by the introduction of more double bonds. Furthermore, EPA could convert into docosahexaenoic acid (DHA) (22:6ω-3) [108].

Arachidonic acid is a fatty acid that should be included in the human diet. It cannot be synthesized by animals or humans, and it plays a crucial role in various aspects of human health, such as brain development during fetal and infant stages, as well as in reducing factors associated with cardiovascular diseases [109], and also in membrane mobility conferring flexibility, fluidity and selective permeability [110].

Eicosapentaenoic and Docosahexaenoic acids also have a significant role in human health as they are associated to preventing or reducing cardiovascular diseases, inflammatory diseases, and cancers [107].

These eicosanoids are powerful lipid mediator signaling molecules that in general when originated from ω-6 PUFA are proinflammatory, while originated from ω-3 PUFA are anti-inflammatory [107]. Normally, ω-6 PUFA arachidonic acids is found in high quantities in human inflammatory cells while having low values of ω-3 PUFA, and the importance of

this difference is that arachidonic acids is a precursor of prostaglandins and leukotrienes, which have significant roles as mediators and regulators of inflammation [108]. In other words, in a plant with inflammation, arachidonic acid works as a signaling molecule that induces stress and defense signaling, which trigger defense mechanisms (eicosanoids derivatives) against the inflammation [111]. Despite contributing to inflammation, the most important aspect regarding ω-6 PUFA arachidonic acids is the generation of mediators that are responsible for the resolution of inflammation and wound healing [110].

In avocado fruit, the PUFA content comprises around 13 % of total fatty acid, with linoleic acid (LA,18:2ω-6) (Fig. 17) being the major one.

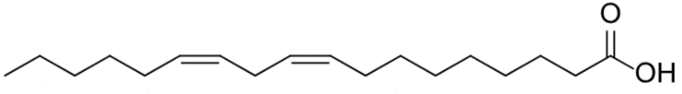


Fig. 17 Polyunsaturated fatty acid – Linoleic acid. Figure from author.

1.8. Health benefits

Over time, the evidence/proofs that the avocado fruit has, or may have, an important beneficial role in human health have grown. So the increase in its consumption, as well as the investigation of it, has been notorious [84]. Many governmental databases from USA, Australia and New Zealand have a standard reference for avocado consumption [16]. Overall, the fruit is associated with the combat against certain diseases.

1.8.1. Anti-cancer properties

Cancer is nowadays one of the worst diseases that we can find, being the one that causes more deaths than malaria, tuberculosis, immune deficiency syndrome and diabetes combined [60]. Its high incidence and mortality rate and the lack of effective treatment has led it to become one of the most studied diseases today. Cancer has the peculiarity of being a disease where mutations are present, associated late diagnosis, expensive therapeutic options, make the creation of anti-cancer regimens difficult and a huge challenge [112]. Vegetables and fruits are associated with several anti-cancer components, which make them, with their consumption, recognized as potential cancer-reducing agents in humans [113].

Avocados contain several bioactive phytochemicals that have been linked to anti-cancer properties, such as carotenoids, terpenoids, D-mannoheptulose, persenone A and B, phenols, and glutathione [8]. The cytotoxicity properties of avocado against different types of cell lineages, breast colon, liver, lungs, larynx, oral, prostate, ovary, oesophageal,

and leukemia have been studied [60]. It's reported that in avocado cancer research, the focal points are cancers affecting the oral cavity, larynx, and pharynx [114].

This is largely attributed to the presence of glutathione, a potent antioxidant tripeptide composed by glutamic acid, cysteine, and glycine [115]. Avocados stand out for their elevated concentrations of this compound compared to other fruits. Other studies have correlated the increase in glutathione intake with a decrease in the risk of cancer in the oral cavity and pharynx [114]. One of the cancers also associated with avocados protection is prostate cancer, closely associated with the presence of lutein, which is known to be anti-proliferative and anti-tumor, being present in high amounts in avocados, which can justified what occurred in a study, where acetonic avocado extracts were put in contact with prostate cancer cells and the inhibition of the profiling of cancer cells was observed [84].

1.8.2. Cardiovascular diseases and diabetes

Another set of diseases that greatly affect society around the world are cardiovascular diseases, including coronary heart disease (CHD) and stroke. It is quite clear that the onset of cardiovascular disease (CVD) is related to the oxidation of cholesterol-rich plasma lipoproteins [116]. This oxidation increases the atherogenicity of lipids facilitating the entry of cholesterol into the arterial wall [117]. The first avocado study related to cardiovascular problems, identified the possibility that avocado can regulate blood cholesterol levels, and the same was verified and proven in other studies, where an avocado diet rich of hypercholesterolemic subjects provoked the decrease of LDL cholesterol and triglycerides levels and the increase of HDL cholesterol levels [8]. Avocado is known for its abundance of unsaturated fatty acids able to promote the proper functioning of the heart as well as the circulatory system, through replacing saturated fats by unsaturated fats. This process has obviously when avocado intake has used to replace to saturated fats like butter, eggs, cheese, reducing the risk of CVD [118,119].

Other problem that is spreaded worldwide is the metabolic syndromes, like the increased obesity, more specific the abdominal obesity characterized by a belly, which this intra-adiposity is related with the increased of type 2 diabetes. This excess of energy is kept in adipose tissues as visceral fat, as well in other organs like liver, skeletal muscle, and pancreas as ectopic fat, which this excess of adipose tissue results in insulin resistance and systemic inflammation [120]. A study carried out on avocado oil revealed that it contributes significantly to the metabolic system, reducing inflammatory responses [121]. Also, its known that avocado intake in meals can cause a satisfactory feeling of being full, reducing

real satisfaction, which indirectly controls the amount of food ingested, causing a decrease in body mass index and consequently the risk of metabolic problems [60].

1.9. Sensory analysis

Sensory perception have a major role in the acceptance of a food products by the consumer, where many studies are based in food attributes such as appearance, taste, aroma, and texture [122]. This analysis can be categorized into two distinct groups, discriminant methods and descriptive methods. Discriminant methods aim to straightforwardly identify differences between tested samples, while descriptive methods, like any chemical analysis, focus on the identification, quantification, and description of sensory attributes in a product by a trained human [123]. These methods used the hedonic tests to performed the analysis [124], which consists in a 9-point Hedonic scale to measure consumer acceptance of food, ranging from level 1- “dislike extremely” to 9- “like extremely” [125].

1.10. Specific objectives

The aim of this study was to try to fill an existing gap by carrying out the first study of avocado bioactive and phytochemical compounds including the analysis of fruit in all tissues (pulp, peel, and seed).

We hypothesize that Madeira regional varieties have a biochemical composition, nutritional value and fatty acid profile, that distinguish them of the Hass commercial variety that on its, performed on Madeira Varieties. The analysis of fruits of regional and commercial varieties has made based on the following points:

- Portugal is one of the greatest avocado producers in Europe, with Algarve and the Madeira Island acting as major producers.
- Regional varieties need reach a wide appreciation since they are constantly being passed over by commercial varieties, leading to their extinction.
- To analysis the by-products of avocado production in relation to its phytochemical properties underscore the importance of studying and exploring potential uses for them.
- To compare regional varieties with commercial ones, in order to determine their similarities and differences.

2 Methods and Materials

In this study, five varieties of avocado (four regional and one commercial) were collected from different farmers and places in Madeira Island (Fig. 18). *Quebrada Grande* (QG) was collected in the Center for Development of Tropical and Subtropical Fruits, at Quebradas, São Martinho, Funchal, (32°38'50.8"N, 16°57'46.4"W, 126 m on altitude), both *Roxa de Casca Fina* (RCF) and *Roxa de Casca Grossa* (RCG) were collected in Santa Cruz (32°42'06.2"N, 16°47'35.8"W, 317 m on altitude and 32°41'53.6"N, 16°47'01.5"W, 182 m on altitude) respectively. *Cabaça* (CB) harvested in Porto da Cruz, Machico (32°45'31.3"N, 16°50'11.1"W, 260 m on altitude), and the commercial variety *Hass* was harvested in Santa Maria Maior, Funchal (32°39'49.2"N, 16°53'40.9"W, 338 m on altitude).

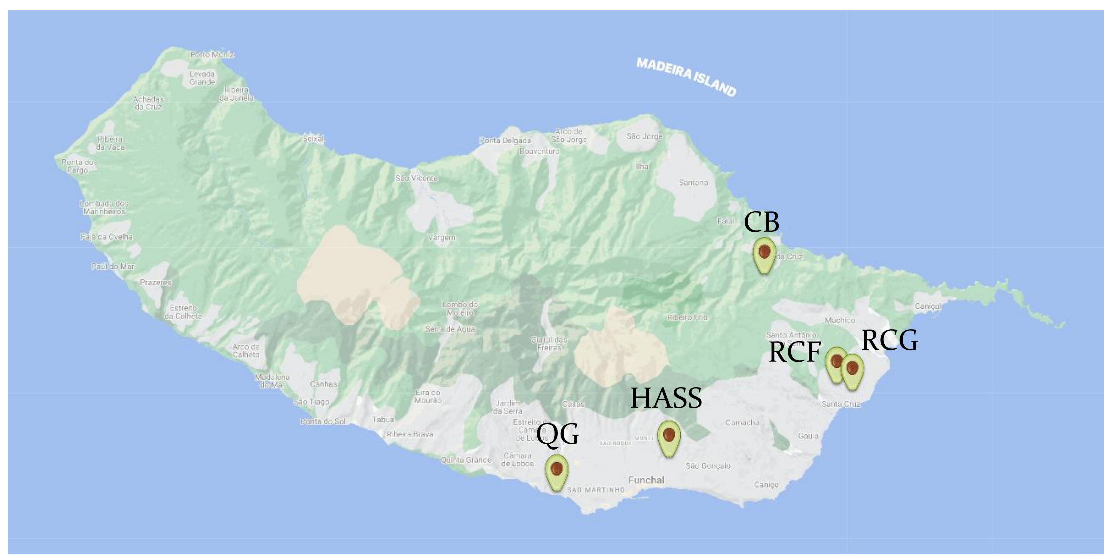


Fig. 18 Geographic visualization of the harvest points of the collected regional avocado varieties. Figure from author.

2.1. Chemicals

Many chemicals were used in the different analyses, all of them with a purity grade higher than 96%.

In physicochemical analysis, sodium hydroxide and phenolphthalein indicator were purchased from Honeywell (Seelze, Germany), and ethanol was from Labchem (Santo Antão do Tojal, Portugal).

In proximate analysis, potassium sulphate, boric acid, sulphuric acid, acetone, ethyl ether, trichloroacetic acid and methanol were purchased from Honeywell (Seelze, Germany). Selenium, hydrogen peroxide, methyl-red indicator, bromocresol-green indicator, celite, folin reagent, sodium carbonate, gallic acid, ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent were from Sigma-Aldrich (Steinheim, Germany). Nitric acid and glacial acetic acid were obtained from Panreac (Barcelona, Spain). Hydrochloric acid, anthrone, absolute ethanol, and starch were from Riedel-de Haën (Seelze, Germany), Termoscientific (Kandel, Germany), Aga (Prior Velho, Portugal) and Merck (Darmstadt, Germany), respectively.

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 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 beginning of 2021, and at the end of 2021 and beginning of 2022, respectively. The third harvest cycle, representing on-tree maturation, the harvest was done one month later than in the second harvest cycle. All the samples were allowed to ripen on a bench.

Ten avocados from the same variety and tree were opened, and the pulp, peel, and seed were separately placed into different containers. The pulp was lyophilized 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 a mechanical convection 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, Oschsenhausen, Germany) at -30°C.

For this work, all samples were analyzed in their dried state, the flour obtained by grinding ten avocados of each variety, along with their respective tissues, was subjected to three measurements for each analysis.

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

2.3. Physicochemical analysis

2.3.1. pH

The pH of the avocado varieties was determined by AOAC [126] method, where 1 g of avocado flour were weighted in an analytical balance (Precisa XR 205 SR-DR, Dietikon,

Switzerland) and mixed with 10 mL of distilled water in a 50 mL tube. The mixture was vortex (Fisher Scientific Topmix FB 15024, Usmate Velate, Italy) for 30 min and left at rest for others 30 min until phases formation.

After that, with a pH meter (WTW PH 320, Weilheim, Germany), readings were performed by inserting the electrode into the aqueous phase of the mixture.

2.3.2. Titratable Acidity

Titratable Acidity was done following the AACC [127] method with some variations, where 3 g of sample (avocado flour) and 30 mL of distilled water, were added to 50 mL tubes. After 30 min in orbital shaker (INNOVA 2100, Edison, New Jersey, USA), the mixture was kept at rest for 1hour. Following that, 17.6 mL of the aqueous phase was collected and added to another 17.6 mL of distilled water in 50 mL beakers. One drop of 2% phenolphthalein was added to each beaker and titration was carrying out with 0,1N NaOH standardized.

The calculation of titratable acidity was done by the following Equation 1,

$$\% \text{ (v/p)} = \frac{[0{,}1N\ NaOH\ spent\ (ml) \times N \times f \times 0.7505]}{[Sample\ weight]} \times 100 \quad (1)$$

with N the 0.1N NaOH concentration, f the NaOH normality correction factor, and 0.7505 the acidity constant of tartaric acid.

2.4. Proximate analysis

2.4.1. Ash

Ash was obtained by following the Portuguese norm NP 518 [128] with some modifications. In 30 mL porcelain crucibles, 1 g of sample was weighed and placed in incineration at 750 ºC for 5 hours in a muffle furnace (Vulcan 3-550, St. Neots, UK). After that, the porcelain crucibles were allowed to cool to room temperature in a desiccator. The ash determination was carried out by gravimetry using an analytical balance. The results were expressed in g/100g of dry weight (DW).

2.4.2. Determination of crude protein

The crude protein content was carried out doing the 945.18-B Kjeldahl method [129] involving three main stages, digestion, distillation, and titration.

The first stage occurs by digesting 1 g of sample with 7 g of potassium sulfate, 5 mg of selenium (these two acts as catalysts) and 12 mL of 96% sulfuric acid (Honeywell) in the

Velp Scientifica DK 8S Heating digester (Usmate Velate, Italy). In digestion, the nitrogen present in the sample was converted into ammonium sulfate, and the interaction between nitrogen and the acid, results in the release of hydrogen (in water molecule H_2O) and carbon (CO_2) (Eq. 2).

$$Organic\ N + H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2 + other\ products \quad (2)$$

The second and third stages took place in the Velp Scientifica UDK 152 distillation and titration unit (Usmate Velate, Italy). During distillation, the ammonium sulphate was neutralized with an excess of 33 % NaOH to convert the NH_4 in NH_3 (Eq.3). The distillate was continually added to a 4 % boric acid (H_3BO_3) solution with the colorimetric indicators - bromocresol green and methyl red (Eq. 4). Finally, the distillate within the boric acid solution was titrated with 0.2 N HCl. The amount of nitrogen present in the sample was converted to crude protein, by multiplying the amount of nitrogen by the conversion factor of 6.25. The crude protein was express in g/100g DW.

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O + CO_2 \quad (3)$$

$$NH_3 + H_3BO_3 \rightarrow + NH_4^+ + H_2BO_3^- \quad (4)$$

2.4.3. Extraction and quantification of soluble sugars

The soluble sugars were extracted and quantified by McCready et al. [130] method with adaptation by Bailey [131], where 100 mg of avocado flour was extracted with 4 mL of heated 80% ethanol, the mixture was centrifuged (Eppendorf centrifuge 5430R, Hamburg, Germany) at 5000 rpm for 10 min, reserving the supernatant. To this, 10 mL of aqueous ethanol was added, centrifuged (with the same conditions), followed with filtration. At the end, the volume of the filtrate was adjusted with distilled water to 50 mL, and stored at 4 °C.

To quantify soluble sugars present in sample, 0.5 mL of the extracted solution was reacted with 5mL of 0.02 % anthrone solution. Then the mixture was placed in a water bath (Julabo SW 22, Seelbach, Germany) for 7 min at 100 °C to break glycosidic bonds and produce furfural compounds. The sulfuric acid present in the anthrone solution breaks down the glucose molecules, giving rise to furfural compounds, which in turn, when in contact with anthrone and heat, react and produce the greenish color, which its intensity can be detected at 620 nm in an UV/Vis spectrophotometer (2401 PC, Shimadzu, Kyoto, Japan), with the UVProbe software. A glucose standard curve (0 to 100 μg/mL) was used

for the quantitation of soluble sugars in avocado flours, with results expressed in g/100g DW.

2.4.4. Extraction and quantification of starch

Starch extraction and quantification was carried out following the Hodge & Hofreiter [132] method with some adaptations. To 15mg of sample (avocado flour), 2.5 mL of 80% heated ethanol was added, followed by centrifugation at 6000rpm for 10min. The supernatant was discarded, reserving the pellet, which is the part where the starch resides. Then, 5 mL of 1.1% HCl was added to the pellet, to hydrolyze the starch into glucose, followed by a water bath at 100°C for 30min, and later by filtration. Of the filtrate, 3.13 mL were removed and added to falcon tubes, with the volume made up to 50 mL with distilled water and stored at 4ºC.

To quantify starch, 1 mL of the extraction solution was reacted with 5ml of 0.2% anthrone solution, in a water bath at 100 °C for 11min, which decomposes the starch into furfural compounds, which produces a greenish color when reacting with anthrone. The quantification was done using a spectrophotometer at 630nm, following a soluble starch standard curve (0 to 10 mg/mL), with results expressed in g/100g DW.

2.4.5. Determination of crude fiber

Fiber was determined using the modified Scharrer method [133] with some modifications. 0.5 g and 1.5 g of celite and sample respectively added to a crucible (P2 Type). After inserting the crucibles in the fiber extractor (Velp Scientific FIWE 6, Usmate Velate, Italy), 30 mL of cold Scharrer reagent (glacial acetic, nitric and trichloroacetic acid) was added and boiled for 30 min. Then, a series of washing was performed, with distilled water, acetone, and ether, to remove all the soluble residue. The crucibles were placed in a ventilated oven (HeraTherm Thermo-Scientific, Waltham, MA, USA) at 130 °C for 1.5 h, to dry the residue (fiber and ash). Lastly, the crucibles with dry residue were weighed, placed in a muffle furnace to incinerate the fiber at 550 ºC for 3 h, following by the weighing the final residue (ash). The results were obtained according to Equation 5 and expressed in g/100g DW.

$$Fiber = [\frac{(Wd-Wf)}{(Wa+Wb+Wc)}] \times 100 \quad (5)$$

where: *Wa* is the sample weight; *Wb* the celite weight; *Wc* is the empty crucible weight; *Wd* is the crucible weight of dry fiber and ashes residue (130 °C, 1.5 h); *Wf* is the crucible weight with ash (550 °C, 3 h).

2.4.6. Determination of chlorophylls and carotenoids

To perform the extraction and determination of photosynthetic pigments, chlorophylls, and carotenoids, it was used the Lichtenthaler [134] method for chlorophylls and total carotenoids, and the conjugation of Biehler et al. [135] and Kizilkaya et al. [136] methods for the total carotenoids and specifics carotenoids like beta-carotene, lycopene and lutein.

In a 15 mL falcon tube, 0.5 g of sample, 1 mL of distilled water and 8 mL of ethanol were added, followed by an overnight incubation in the dark. Then, the extract was centrifuged at 6000 rpm for 10 min, keeping the supernatant. In Lichtenthaler [134], the pigments were detected using a spectrophotometer at 648.6 nm and 664.2 nm for chlorophylls (Eq. 6-8), and 470 nm to total carotenoids (Eq. 9). In Biehler et al. [135] and Kizilkaya et al. [136], using acetone instead of ethanol and using the same procedure of extraction, the carotenoids were detected at 445, 449, 452 and 448 nm, for lutein (Eq. 10), total carotenoids (Eq. 11), beta-carotene (Eq. 12), and lycopene (Eq. 13) respectively. Results were expressed as mg/100g DW.

Chlorophyll *a*:

$$\mathrm{C} = \frac{(13.36\ A664.2 - 6.19A648{,}6) * 8.1}{DW} \tag{6}$$

Chlorophylls b:

$$\mathrm{C} = \frac{(27.43A648.62 - 8.12A664{,}2) * 8.1}{DW} \tag{7}$$

Total Chlorophylls:

$$\mathrm{C} = \frac{(5.24\ A664.2 - 22.24A648{,}6) * 8.1}{DW} \tag{8}$$

Total Carotenoids:

$$\mathrm{C} = \frac{(4.785\ A470 - 3.657A664{,}2 - 12.76A648.6) * 8.1}{DW} \tag{9}$$

Lutein:

$$\mathrm{C} = \frac{A445 * Fd}{135310} \tag{10}$$

Total Carotenoids:

$$\mathrm{C} = \frac{A449 * Fd}{135310} \tag{11}$$

Beta-carotene:

$$C = \frac{A452 * Fd}{135310} \tag{12}$$

Lycopene:

$$C = \frac{A448 * Fd}{135310} \tag{13}$$

In these equations, DW refers to Dry weight, Fd was the dilution factor, and 135310 is the average molar absorption coefficient.

2.5. Colorimetric analysis of avocado flour

Colorimetric analysis of the avocado flour from pulp, peel, and seed was determined by reflectance using a X-RITE Ci-7600 Sphere Benchtop Spectrophotometer (MI, USA), using the CIE L*a*b* color space system as reference, based on a light incidence angle of 10° and illuminant D65.

2.6. Determination of antioxidant activity

Antioxidant activity was assessed using a method slightly modified from Fan et al. [137], incorporating elements from Rocchetti et al. [138], 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 Velate, 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. Finally, following centrifugation at 5,000 rpm, at 10 °C for 15 min, the supernatant was stored at -20 °C.

2.6.1. 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), with results expressed in mg GAE/g DW.

2.6.2. 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), with results expressed in mg AAE/g DW.

2.7. Lipids extraction and quantification

The extraction and determination of lipid content in avocado flours were conducted based on the AACC [139] 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, also positioned within the bottom of the apparatus. The extraction process took 4h, after which the excess solvent was removed using a rotary evaporator (Heidolph, Sxhwabach, 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 until a constant weight was achieved, then moved to a desiccator, 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. [140], 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-5J&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 DW.

2.8. Index of Atherogenicity (IA) and Index of thrombogenicity (IT)

To assess the nutraceutical value associated with fatty acids, calculations based on Chen & Liu [141] were performed. These calculations determined the atherogenicity index (Eq. 14), which evaluates the lipid adhesion to cells in the circulatory and immunological systems, and the thrombogenicity index (Eq. 15), which assesses the propensity to form blood clots in vessels.

$$IA = \frac{[C12:0 + (4 \times C14:0) + C16:0]}{\Sigma UFA} \tag{14}$$

$$IT = \frac{[C14:0 + C16:0 + C18:0]}{[(0.5 \times MUFA) + (0.5 \times \omega 6PUFA) + (3 \times \omega 3PUFA) + \left(\frac{\omega 3}{\omega 6}\right)]} \tag{15}$$

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

2.9. Sensory Analysis

Sensory analysis (ISO 4121:2003; ISO 6658:2017)[142,143] of the pulp flour was carried out by the AgroSenseLab - a sensory panel of trained tasters for agricultural and agri-food products from the Regional Secretariat for Agriculture and Rural Development of the Autonomous Region of Madeira. For the analysis, the pulp samples collected at the regular harvest time were sent to the panel of tasters, along with identical samples from the late harvest.

2.10. 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 (HSD). Statistical differences with $p \leq 0.05$ were considered significant. The principal component analysis (PCA) was performed using MVSP V3.1 statistics program.

Avocado pulp is renowned for its nutritional value and vitamins and phytochemicals abundance, that contribute to promoting good health. However, it is important to acknowledge that avocado by-products also contain remarkable bioactive properties that could potentially offer health benefits to humans [137,144]. To the best of our understanding, this dissertation for the first time examines nutritional and antioxidant properties and describes the fatty acid profile of four local avocado varieties from Madeira Island. This analysis was performed in all tissues (pulp, peel, and seed), and the regionals varieties were compared with the Hass commercial variety across different cycles.

3.1. Physicochemical analysis

3.1.1. pH and Titratable Acidity

When we talk about physicochemical analysis, pH and titratable acidity are two parameters that we almost instantly think, even more so when food samples analysis for are considered [145]. These two parameters are interconnected and have their own influence on food quality [145].

In the pH level analysis, it is notable that in the majority of the samples, the pulp shows the highest values, followed by peel and seed. In the first harvest cycle, the pulp showed values ranging from 5.94 to 6.44, while in the peel was observed values spanning from 5.33 to 6.18 and, lastly, the seed revealed values varying between 4.88 to 5.62.

The second harvest cycle shows little variation in the pH values. In the pulp, a decreased of pH values was observed in the most of regional varieties, apart from the RCF variety, which increased its value. The values obtained by regional varieties, varying between 5.66 to 6.37, and the Hass variety recorded the highest value in 6.69. Related to peel and seed, during this harvest cycle, values ranged from 5.15 to 5.90 and 4.96 to 5.88, respectively. As for the peel, half of the samples increased their values, while the other half decreased their values. Conversely, in the seed, almost every sample increased its values. It

is worth highlighting that *Hass* variety, in accordance with pulp, revealed the highest values with 6.01 in peel, and 6.07 in seed.

About the third harvest cycle, in all tissues, most samples revealed an increase in the pH values. The obtained values ranging from 6.23 to 6.69 in pulp, 5.16 to 5.86 in peel, and 5.22 to 5.49 in seed. The values of the *Hass* variety were of 6.36, 6.09, and 6.09, in pulp, peel, and seed. In general, the commercial variety was the one with higher pH values, in all tissue and harvest cycles analyzed, while in the regional cluster of varieties, QG variety was the one with higher values in the majority of tissues and harvest cycles. Conversely, CB variety was the one with lowest pH values in the same conditions.

Our results are in line with those found in the literature, where Kassim & Workneh [146] observed a variation in pH values in the pulp, under different storage condition ranging from 6.03 to 6.76. Similar, Astudillo-Ordónez & Rodríguez [147] analyzed pulp in different harvest points, and displayed values spanning from 6.58 to 7.14, slightly higher than ours. Another study obtained values slightly higher than ours, ranging from 6.18 to 7.25 [148]. In comparison to ours, Tan et al. [149] analyzed the physicochemical properties of red avocado in all tissues (pulp, peel, and seed), obtaining values in pH analysis of 5.48 in pulp, 5.04 in peel, and 5.59 in seed, which is lower than our findings in pulp, and peel, but similar to our seed values. Related to the peel, Calderón-Oliver et al. [150] obtained values from peel extracts ranging from 5.46 to 6.48, which, in general, are values similar to ours. Nyakang'I et al. [151], in his study about the use of seed extracts in beverages and the use of seed powder in baked products, achieved a pH value of 5.5, which is easily related to our findings. In other study, Buelvas et al. [152], demonstrated the correlation between the state of maturation and pH, where the greater the maturation, the higher the pH, which is clearly evident in our results, especially from the 2nd to the 3rd harvest cycle.

Navigating through the Table 2 by line, we could state that statistically significant differences ($p<0.05$) were observed, in first harvest cycle, all varieties were different in pulp tissue, except the RCF and RCG varieties that were equal. Peel and seed displayed significant differences in all varieties. In the following harvest cycle, the pulp and peel showed the same pattern, where all varieties were different, apart from RCG and CB that demonstrated equality. Seeds, almost follow this pattern, with CB being similar to RCG, and RCF. In the last harvest cycle, more variability was detected within the pulp and the peel. In the first case, RCF and RCG were equal, but statistically different from CB, and both QG and *Hass* were also equal, and similar to the other ones. In the peel, RCF, RCG and *Hass* are different from each other, with the other two varieties displayed equality, and similarity with the RCG and *Hass*. RCF was statistically different from all the others. Contrary to these two tissues,

in the seed, all regional varieties demonstrated equality, with the *Hass* differing from all the others. Following the capital letters along the column, statistical differences were verified within each variety and in each tissue along cycles. The pulp and seed in QG variety revealed the same content, where all harvest cycles presented differences and, in the peel, the first harvest cycle was significantly different from the other two. In the pulp, in RCF variety, the first and third harvest cycles revealed differences, being the second harvest cycle similar to them. Among the by-products of this variety, no differences were observed in seed, but the peel of the first harvest cycle showed distinctions in relation to the other cycles. The RCG variety shows differences in the pulp of the all three harvest cycles. Also, the peel of the three harvest cycles shown to have similarities in spite of their variation. The pH of seeds of first harvest cycle reveals differences in relation to the other cycles. In the pulp of CB variety, differences were detected in all harvest cycles, and the peel and seed followed the same pattern, where the third harvest cycle displayed differences in relation to the other cycles. *Hass* variety showed no statistical differences between the harvest cycles.

3.2. Titratable Acidity (TA)

In our TA analysis, statistical differences between the tissues were evident, where the pulp was the one that resisted higher values, followed by the seed and finally the peel. In the first harvest cycle, our pulp levels ranged between 0.55 to 0.86% of tartaric acid, while peel and seed obtained values ranging from 0.31 to 0.36% and 0.33 to 0.50%, respectively, for tartaric acid. Moving on to second harvest cycle, the majority of the samples decreased its values of TA in all of the three tissues, where in the pulp were observed the increase in two varieties, and decreased in the other two, spanning the tartaric acid from 0.45 to 0.99%, meanwhile 0.26 to 0.57% were registered in peel, and 0.27 to 0.43% in seed. In the final harvest cycle, were observed an increase in almost all tissues of every variety in all tissues, where the tartaric acid values ranged between 0.62 to 0.84% in the pulp, followed by 0.27 to 0.60% in the peel, and finalizing with 0.29 to 0.50% in the seed. Notably, in general, the QG and CB varieties were the ones with higher TA values, while RCF and RCG were the lowest.

In one study performed by Salameh et al. [153], the pulp of seven varieties cultivated in Lebanon obtained very low TA values when related to ours, ranging from 0.08 to 0.18%. Another study demonstrated that TA values in the pulp of six varieties cultivated in Egypt, spanning from 0.83 to 0.89%, which were slightly higher than ours [154]. Krumreich et al. [155] studied the physicochemical characteristics and bioactive compounds in the avocado pulp of the Breda variety and obtained a value of TA of 0.53%, which is lower than our

findings. In a different pattern of study, Tan et al. [149] displayed an analysis in all tissues of Thompson red avocado fruit, were showed TA values of 0.27% for the pulp, and 0.42% for both peel and seed, which is much lower, related to most of our samples.

The variability in the parameter could be associated with to the location where the avocado was cultivated, more precisely, the different locations altitude [153]. This was not the case with our results, because our lowest elevation variety (QG) predominantly yielded the highest TA values. Conversely, *Hass* cultivated at highest elevation did not consistently yield the lowest values. On the other hand, it is been stated in literature that the maturation stage could be related with the decreased in the acidity content due to the use of organic acids during respiration in the ripening process [152]. Again, it was not verified in our samples, easily justified by the fact that our 3rd harvest cycle was not a maturation on the tree but rather a late harvest. As a food quality parameter, it was suggested by Athmaselvi et al. [156] that low TA values (≤0.50%) make more perishable the food, which our results, although low, are mostly (mainly the pulp) above this threshold.

In general, our TA values are in line with the values described in the literature, since the avocado fruit is classified as a non-acidic fruit, where its organic acids are mainly tartaric acid and trace residues of citric and malic acid [17,154].

Following the same exercise in pH analysis, scrolling through the Table 2 by line, we could state that statistically ($p<0.05$), in the first harvest cycle of pulp, the QG variety differs from all the other. Meanwhile, all the other varieties were interconnected, with RCG and CB displayed statistically differences, while RCF resembled both. The by-products almost showed no differences among the varieties, in the same harvest cycle, with the only exception belonging to the peel, where the RCF variety statistically differed from all the other varieties. More variability in the TA values was detected in the second harvest cycle.

In the pulp, most of the varieties showed differences with each other, with only RCG variety showing resemblance to QG variety.

The peel of *Hass* statistically differed from peel of all the other varieties. Meanwhile, two clusters were detected, first joining the QG and RCF varieties and the second the RCG and CB varieties. The TA peel of QG, RCF, and RCG showed similarity. The CB and *Hass* varieties differed statistically from each other and from the remaining varieties.

In the seed, were detected statistical differences between varieties, excluding the *Hass* variety who showed similarity with RCF and RCG varieties. In the seed, two clusters were detected with QG and CB, showing similarity, and with RCF, RCG, and *Hass* varieties, filling the other group.

Going through the capital letters, QG variety showed different statistical behavior in all the three tissues, with differences in all the three harvest cycles in the pulp, followed by a difference in the first harvest cycle related to the other two cycles in the peel, and the similarity of the second harvest cycle with the others two cycles (that differed from each other) in the seed. In RCF variety, the first and third harvest cycle showed similarity with the second, differing from them in the pulp. In the peel, no significant differences were detected in the harvest cycles, while the first cycle of the seed displayed variations compared to the other cycles. Moving on to RCG variety, differences were observed between the first and third harvest cycle in the pulp, with the resembled of the second cycle to both. The by-products revealed the same pattern, with one cycle differing from the others, which in the peel case, was the third cycle. Meanwhile, the first harvest cycle was the one in the seed case. In the CB variety, all the three harvest cycles presented differences in the pulp. Conversely, only the first harvest cycle showed variations in the peel. In the seed, the second and third harvest cycles showed differences, with the first cycle demonstrating similarity with both. Passing through the *Hass* variety, differences were detected only in the pulp.

Table 2 Physico-chemical analysis in five different avocado varieties.

PA	Tissue	Cycle	Varieties QG	RCF	RCG	CB	Hass
pH	PULP	1st	6.44 ± 0.02 ^{a,A}	6.22 ± 0.03 ^{b,A}	6.15 ± 0.04 ^{b,A}	5.94 ± 0.06 ^{c,A}	na
		2nd	6.16 ± 0.02 ^{a,B}	6.37 ± 0.08 ^{b,AB}	5.76 ± 0.05 ^{c,B}	5.66 ± 0.01 ^{c,B}	6.69 ± 0.13 ^{d,A}
		3rd	6.59 ± 0.05 ^{ab,C}	6.66 ± 0.20 ^{b,B}	6.69 ± 0.13 ^{b,C}	6.23 ± 0.08 ^{a,C}	6.36 ± 0.16 ^{ab,A}
	PEEL	1st	6.18 ± 0.01 ^{a,A}	5.61 ± 0.00 ^{b,A}	5.42 ± 0.04 ^{c,A}	5.33 ± 0.03 ^{d,A}	na
		2nd	5.90 ± 0.06 ^{a,B}	5.15 ± 0.06 ^{b,B}	5.58 ± 0.04 ^{c,AB}	5.55 ± 0.03 ^{c,A}	6.01 ± 0.20 ^{a,A}
		3rd	5.86 ± 0.06 ^{ac,B}	5.16 ± 0.01 ^{b,B}	5.70 ± 0.14 ^{a,B}	5.86 ± 0.15 ^{ac,B}	6.09 ± 0.08 ^{c,A}
	SEED	1st	5.62 ± 0.01 ^{a,A}	5.09 ± 0.01 ^{b,A}	4.88 ± 0.01 ^{c,A}	5.04 ± 0.01 ^{d,A}	na
		2nd	5.88 ± 0.02 ^{a,B}	4.96 ± 0.02 ^{b,A}	5.29 ± 0.05 ^{c,B}	5.13 ± 0.03 ^{bc,A}	6.07 ± 0.14 ^{d,A}
		3rd	5.27 ± 0.01 ^{a,C}	5.22 ± 0.17 ^{a,A}	5.32 ± 0.04 ^{a,B}	5.49 ± 0.11 ^{a,B}	6.09 ± 0.13 ^{b,A}
Titrable acidity (%)	PULP	1st	0.86 ± 0.01 ^{a,A}	0.63 ± 0.05 ^{bc,A}	0.55 ± 0.05 ^{c,A}	0.70 ± 0.05 ^{b,A}	na
		2nd	0.66 ± 0.03 ^{a,B}	0.45 ± 0.03 ^{b,B}	0.60 ± 0.03 ^{ac,AB}	0.99 ± 0.04 ^{d,B}	0.55 ± 0.04 ^{c,A}
		3rd	0.79 ± 0.03 ^{ac,C}	0.62 ± 0.06 ^{b,A}	0.68 ± 0.04 ^{b,B}	0.84 ± 0.03 ^{c,C}	0.70 ± 0.03 ^{ab,B}
	PEEL	1st	0.36 ± 0.01 ^{a,A}	0.47 ± 0.02 ^{b,A}	0.31 ± 0.04 ^{a,A}	0.34 ± 0.02 ^{a,A}	na
		2nd	0.43 ± 0.03 ^{a,B}	0.46 ± 0.03 ^{a,A}	0.28 ± 0.03 ^{b,A}	0.26 ± 0.01 ^{b,B}	0.57 ± 0.03 ^{c,A}
		3rd	0.47 ± 0.03 ^{a,B}	0.46 ± 0.05 ^{a,A}	0.41 ± 0.03 ^{a,B}	0.27 ± 0.03 ^{b,B}	0.60 ± 0.04 ^{c,A}
	SEED	1st	0.33 ± 0.07 ^{a,A}	0.42 ± 0.05 ^{a,A}	0.50 ± 0.05 ^{a,A}	0.46 ± 0.04 ^{a,AB}	na
		2nd	0.43 ± 0.01 ^{a,AB}	0.27 ± 0.01 ^{b,B}	0.32 ± 0.01 ^{c,B}	0.37 ± 0.01 ^{d,A}	0.30 ± 0.01 ^{bc,A}
		3rd	0.48 ± 0.03 ^{a,B}	0.31 ± 0.03 ^{b,B}	0.31 ± 0.01 ^{b,B}	0.50 ± 0.04 ^{a,B}	0.29 ± 0.04 ^{b,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 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 tissue, in the distinct harvest cycles (years). na- not analysed.

3.3. Proximate Composition

3.3.1. Crude Protein

Even though that total protein is not a very influential parameter when we talk about avocado, it is known that in addition to fat (lipid portion), avocado is a fruit rich in proteins, being one of the fruits with the highest concentrations [60], accounting for around 10% of dry matter [157].

In the first harvest cycle of the protein parameter, it became evident that the pulp was the tissue with the highest protein content, followed by the peel and the seed. Protein levels variated, in dry weight content, from 4.11 to 7.44 g/100g in the pulp, 2.97 to 7.02 g/100g in the peel, and 2.48 to 4.55 g/100g in the seed. Advancing to the second harvest cycle, a slightly increase was visible in both the pulp and seed tissues, with values ranging from 4.92 to 10.54 g/100g, and 2.61 to 5.23 g/100g, respectively. Meanwhile, a decrease was observed in the peel, with values spanning from 2.74 to 6.89 g/100g. Moving on to the third harvest cycle, most of the samples decreased its protein values in the pulp tissue, contrarily to the peel and seed, that with the same terms increased its values. In the pulp, values were obtained between 4.58 to 7.80 g/100g, while peel and seed showed values ranging from 2.71 to 7.01 g/100g and 2.53 to 5.51 g/100g, respectively.

Many literature indicated levels of protein lower than our findings [60], quoting the United States Department of Agriculture, that revealed the value of protein in the pulp of avocado of 1.96 g/100g (FW). Other study showed values of protein in all three tissues of the avocado fruit, with 1.82 g/100g in the pulp, while the by-products displayed values of 1.91 g/100g and 2.19 g/100g for the peel and seed, respectively [39]. Notably, in this study, contrarily to ours, the tissue with highest protein content was the seed, followed by the peel and pulp. Souza Jorge et al. [158] in their study, the pulp of two commercial varieties of avocado obtained values of 7.17 g/100g in *Margarida* variety and 5.66 g/100g in *Hass* variety, which are slightly higher than our values. In another study performed by Blakey et al. [159], they analyzed a series of proximate parameters in the commercial *Hass* variety, where a distinction was made through fast and slow ripening process. The protein values oscillated between 1.53 to 3.37 g/100g in the slow rate, and 1.77 to 3.55 g/100g in the fast-ripening rate, which indicated that fast ripening process increased the protein values. Another study performed in the avocado by-products indicated values of protein between 4 to 8.3 g/100g in the peel, and values of 4.8 g/100g in the seed, which is related to our results [144].

Focusing on the statistical analysis ($p \leq 0.05$) of the protein analysis and going through Table 3 line by line, we observed that all varieties, and all tissues, displayed differences in the first harvest cycle. Moving to the second harvest cycle, in the pulp, QG and RCF varieties showed similarity, while RCG and CB displayed statistical differences to all. *Hass* variety resembled QG, RCF, and CB varieties. The peel of QG and *Hass* varieties presented similarity, and the same was observed between RCG and CB varieties. Meanwhile, RCF variety differed from all varieties. The seed protein content of RCF, RCG, and CB varieties showed differences between them, while QG and *Hass* resembled each other and with RCG and CB.

The pulp of QG, RCF, and *Hass* varieties in the final harvest cycle resemble together, while the CB variety differed from all varieties, except the RCG variety who revealed similarities with all varieties. In the peel, all varieties are different, except the QG variety, which showed similarity with RCG variety. Meanwhile, in the seed, all varieties revealed variations between them.

Looking through the capital letters, where statistical differences were analyzed within each variety, in each tissue along the harvest cycles, the QG variety showed similarity in all the three cycles for pulp, contrarily to the peel and seed, which displayed differences in all harvest cycles. The same pattern was followed by the RCF variety, in which pulp and seed presented differences in two harvest cycles, and peel revealed similarities in one harvest cycle. The RCG variety exhibited variations in pulp across all the harvest cycles. However, in the peel, the first and third harvest cycles differed, while the second cycle resembled both. In the seed, only the first harvest cycle displayed differences from the others. In contrast, the CB and *Hass* varieties demonstrated completely opposite patterns in this statistical analysis. The CB variety exhibited differences in all tissues and harvest cycles, while the *Hass* variety showed similarity in all tissues and cycles.

3.3.2. Fiber

The avocado fruit stands out for having between 65-85% of dietary fiber, which consists of a mixture of soluble and insoluble fibers, as is the case with cellulose, hemicellulose and pectin, and stands out even among fruits and vegetables, as the highest in fiber content [47].

Our results, exhibited an interesting behavior, since the higher values of fiber were mostly found in the peel, while only trace amounts of fiber were detected in the pulp and seed. Variations in the pulp content were observed across the different harvest cycles. In the initial harvest cycle, the pulp content ranged from 0.30 to 1.04 g/100g. Moving on to the

second harvest cycle, most of the varieties either maintained or increased their pulp content, ranging from 0.30 to 0.61 g/100g, with the exception of a decrease in the QG variety. In the third harvest cycle, a notable shift occurred compared to the previous harvest cycle. Here, nearly every variety, either maintained or reduced its pulp content, ranging from 0.29 to 0.92 g/100g, with the exception of an increase in the CB variety.

In the peel, it was evident the pattern followed by the varieties related to the harvest cycles. In the first cycle values ranges from 9.87 to 34.74 g/100g, while in the second cycle, an increase was detected in all varieties, with values spanning between 23.56 to 36.12 g/100g. In the last cycle, the values obtained ranged from 22.30 to 35.96 g/100g, stating a reduction in the fiber content. It is important to highlight that, in both the pulp and peel tissues, the CB consistently displayed the highest fiber content across all harvest cycles.

At the seed, in the first harvest cycle, the observed values ranged from 0.19 to 0.25 g/100g. However, in the second harvest cycle, a combination of outcomes emerged, with two varieties experiencing an increase in fiber values and two varieties a decrease, with values that ranged between 0.16 to 0.23 g/100g. In the third cycle, most of the varieties are maintain or decrease, oscillating from 0.15 to 0.22 g/100g.

Relatively, Salazar-López et al. [160] stated that the portion of fiber between avocado tissues ranged between 1.4 to 3% in the pulp, 1.3 to 55% in the peel, and 2 to 4.2% in the seed, which is relatable with our finding. In the same pattern, Dominguez et al. [161] referred higher values of fiber in avocado tissues, namely 6.8% in the pulp, while in the peel and seed values ranged from 50.7 to 51.9%, and 7.3 to 9.1%, respectively. However, other studies shown values similar to ours, since described its values ranging from 1.4 to 3% in the pulp, while the by-products stayed in the 50% and 3.9% in the peel and seed, respectively [162].

Statistically speaking the same exercise that was performed for the previous parameters, (Table 3). In the first harvest cycle, only one variety shows differences, in pulp and seed, to all the other, namely the QG variety for the pulp and RCG for the seed. In the peel, all the varieties significantly displayed differences. Moving to the second harvest cycle, in the pulp, two main groups were formed between the regional varieties, with the QG and RCF showing to be similar, and RCG and CB following the same pattern. All regional varieties are different from the commercial variety *Hass*. In the peel, all varieties revealed significant differences, with only RCG and CB varieties displaying to be similar. In the seed, QG and CB varieties resembled each other, while RCF displayed similarities with both RCG and *Hass* varieties. On the other hand, RCG showed similarities with QG, CB, and RCF. In the last harvest cycle, exactly opposite paths were followed by the pulp and peel, since no

differences were identified between varieties in the first tissue, while only differences were observed in all varieties in the second tissue. In the seed, differences were observed between the regional varieties, with the RCF variety showing similarities with the *Hass* variety.

Following the capital letter by column-wise, we observed that the fiber in QG variety shows the same behavior in all the three tissues and harvest cycles, with the first cycle to be distinguish from the other two. In the case of the RCF variety, there were no notable differences in pulp among the harvest cycles, but the story was different when it came to the by-products, where relative differences were evident across all harvest cycles. In the RCG variety, the pulp and peel showed a variation in the second harvest cycle compared to the others, while the seed displayed differences in all cycles. In the CB variety, there were minimal variations between the harvest cycles in all tissues, except for the peel of the first cycle, which deviated from the other. In the *Hass* variety, was found differences only in the seed.

3.3.3. Ash

The ash content, inorganic residue that results from samples incineration, depends of the quantity and composition of the food [163].

In our analysis, it was encountered slightly variations of total mineral content between the harvest cycles in all the tissues, except for one or two cases, when the variation was high, and the pulp assumed the role of the tissue with the highest ash content, followed by the skin and finally the seed.

In the first harvest cycle, values in dry weight related to the pulp ranged between 2.65 to 6.86 g/100g, while the by-products spanned from 2.66 to 3.68 g/100g in the peel and 1.81 to 3.08 g/100g in the seed. Moving on to the second harvest cycle, most of varieties increased its ash values, with the pulp achieving values that ranged between 3.10 to 6.92 g/100g, and both peel and seed obtained values oscillating between 1.58 to 6.47 g/100g and 1.62 to 3.11 g/100g, respectively.

Contrarily to the second harvest cycle, the last cycle showed in general a decrease of mineral content in most of the varieties, with the pulp lowering its values from 2.65 to 6.49 g/100g, while the by-products went between 2.23 to 6.66 g/100g in the peel, and 1.92 to 2.97 g/100g in the seed. It is important to highlight that in this parameter, the QG variety was the one that recorded the highest values in majority of all harvest cycles and tissues.

Relatively to our results, a proximate analysis of *Hass* fruits cultivated in Algarve, shown ash values of 1.77%, 1.50%, and 1.29% in the pulp, peel and seed, respectively, which were lower than ours [39]. Similarity, Krumreich et al. [155], performed the same analysis in

pulp of *Breda* variety, exhibiting ash values of 0.6%, which was much lower than our findings. The pulp from the avocado cultivated in Ecuador, using sustainable practices, it displayed values of ash ranging from 8.90 to 10.44 g/100g, which were slightly higher than ours [164]. Other study by Domínguez et al. [161] published values of ash similar to ours, with 1.77% in the pulp, 3.9% in the peel, and 3.4% in the seed.

When examining the statistical analysis of the Table 3 line by line, it becomes evident that both pulp and seed followed a similar trend. Most varieties displayed an increase in ash content during the second harvest cycle, and a decrease in the last harvest cycle. However, the peel exhibited an opposite pattern, with a decrease in the second harvest cycle, followed by an increase in the third harvest cycle.

During the first harvest cycle, all tissue types showed variations among the different varieties, with only two showing similarity. In the cases of the pulp and seed, RCF and RCG have similar content, while for the peel, it was the RCF and CB. The following harvest cycle revealed a more pronounced difference, with the pulp showing differences in all the varieties, while in the peel, only RCF and CB varieties displayed similarity. In the case of seeds, we observed more similarities, particularly between the QG and CB, when the RCG shows to be closer to *Hass*.

The third harvest cycle, in the pulp, only the RCG exhibited similarity with the other varieties, in this case with the QG and *Hass*. Meanwhile, the by-products revealed differences between all the varieties.

Moving on to a statistical analysis (as shown in Table 3) ($p \leq 0.05$) and utilizing capital letters to represent columns for differences of into each variety and tissue, and between harvest cycles, it was notorious some variances. The QG variety displayed a different variation in each tissue along the harvest cycles. The pulp contrasts with the seed because the differences obtained in all harvest cycles in the first tissue, contrasts with the similarity registered in all harvest cycles in the second tissue. The first cycle of the peel displayed differences to both the other harvest cycles. In the RCF variety, the pulp and seed revealed the same ending with the second harvest cycle that differed from the others, while in the peel, the different harvest cycle was the first one. Both RCG and CB varieties displayed differences between all harvest cycles in each tissue. Conversely, in the *Hass* variety, only the peel showed significant differences between the harvest cycles.

3.3.4. Total Sugars

Regard to total sugar content its evident the several variances, which is in line with other analyzes, between the cycles and the varieties. Our first harvest cycle displayed values,

in dry weight, that ranged between 0.72 to 5.87 g/100g, while the by-product's values ranged from 1.47 to 2.90 g/100g for the peel and 2.30 to 7.38 g/100g for the seed.

In the second harvest cycle, the pulp revealed a widespread increase in sugar content in the varieties, with values ranging from 1.39 to 9.20 g/100g. The same happened in the peel, with values ranging from 2.25 to 5.05 g/100g. Conversely, in the seed, a decreased in the generality of varieties were detected, with values ranging from 2.14 to 4.06 g/100g.

Moving on to the last harvest cycle, a decrease was observed in most varieties for the pulp and seed, with values ranging from 1.24 to 5.71 g/100g and 1.70 to 5.99 g/100g, respectively. In the peel, three varieties raised their values, while the other reduced theirs, with values ranging between 1.10 to 8.86 g/100g.

In literature, the are many studies that analyzed this parameter in avocado, like Viera et al. [164]. In his study of phytochemicals in avocado cultivated under sustainable agriculture practices, they obtained values in the pulp ranging from 3.55 to 4.67 g/100g. In another study by Stephen & Radhakrishnan [162], they indicated that sugars accounted for 0.3g/100g in the pulp of avocado, which are much lower than our results. Another study performed in the physicochemical characteristics of the avocado pulp from the *Breda* variety showed 6.4% of total sugars, which is comparable to ours findings [155]. In a study conducted on the proximate compositions of the pulp, peel, and seed of the *Hass* variety, they observed values of 0.26, 0.65, and 1.64 g/100g, respectively, which were much lower than our results [165].

Fluctuations in sugar concentrations, encompassing both increases and decreases, can be attributed to various factors, primarily owing to the climatic nature of this fruit, where sugar composition varies with the seasons [166].

An essential factor contributing to these variations is the composition of sugars in avocados, primarily consisting of C7 and C6, with a predominant presence of C7. These sugars tend to be converted into energy for the plant as it matures, ultimately leading to a decline in sugar levels. This decline became evident in our third cycle, coinciding with the late harvest [166].

After a detailed examination of the data presented in Table 3 row-by-row, it is possible to detected differences ($p \leq 0.05$) in the first harvest cycle of the pulp and seed, where all varieties displayed differences between them. In the peel, QG and RCG varieties showed similarity, just as the RCF and CB varieties did. Moving to the second harvest cycle, in the pulp, only the QG and RCF varieties exhibited similarity, while in the peel, a big group composed by RCF, RCG, and *Hass* displayed similarity. In the seed, the RCF shows to be like *Hass*. In the last harvest cycle, in the pulp, all varieties exhibited significant differences,

except for the RCF and *Hass* varieties. In the peel, all varieties showed significant differences. Conversely, in the seed, the RCF, RCG, and *Hass* varieties obtained similarity.

When we conducted the statistical analysis ($p \leq 0.05$) by examining the data column-wise, for differences of into each variety and tissue between the harvest cycles, it was notorious huge variances. The QG, RCF, CB, and *Hass* varieties displayed differences in all tissues and harvest cycles, highlighting the enormous variability that exists in each variety, while RCG variety demonstrated different patterns in each tissue, with the second harvest cycle revealing differences against the others in the pulp. In the by-products, the third harvest cycle showed differences to the others, while all cycles displayed differences in the seed.

3.3.5. Starch

The content of starch in avocado fruit is only and exclusively of the seed part, that it is known to be the reserve tissue [167].

The first harvest cycle, the values, in dry matter, oscillated between 35.43 to 41.26 g/100g, followed by a decrease in the second harvest cycle, where the values ranged from 19.33 to 36.95 g/100g. Lastly, in the seed, it was verified a raise of the starch values, ranging between 29.40 to 35.48 g/100g.

Salazar-Irrazabal et al. [167] obtained the starch content of three commercial avocado varieties, such as, *Criolla*, *Fuerte*, and *Hass*, with values of 17.79%, 15.91%, and 28.18%, respectively, which were lower than our findings. Similarity to our results, Wang et al. [168] extracted starch from eight avocado cultivars, where he obtained a yield ranging from 12.6 to 26.3%. Other study illustrated the starch content of seed in *Hass* variety, with values of 41.8 g/100g, which was slightly higher than our results [169].

When examining the statistical analysis of Table 3, it becomes evident that great variability is present in this parameter. In the first harvest cycle, QG and RCF varieties showed similarity with themselves, and with both the other varieties. Moving to the second harvest cycle, both RCG and CB varieties displayed similarity with themselves and with the QG and RCF varieties, while the commercial variety *Hass* differed from all. In the final harvest cycle, RCF displayed similarity with all varieties, whereas RCG and CB varieties not only demonstrated similarity but also more resemblance to other varieties. Specifically, RCF exhibited similarities with the QG variety, and the CB variety shared similarities with the Hass variety.

When we conduct a statistical analysis ($p \leq 0.05$) by examining the data column-wise for differences of into each variety between cycles, it was notorious the huge variances,

with only the *Hass* variety displaying similarity between the cycles. Meanwhile, the other varieties exhibited differences in all the harvest cycles.

Table 3 Nutritional analysis (g/100g) in five different avocado varieties.

PA	Tissue	Cycle	Varieties QG	RCF	RCG	CB	Hass
Protein	PULP	1st	7.44 ± 0.01 [a,A]	4.77 ± 0.01 [b,A]	4.11 ± 0.03 [c,A]	5.10 ± 0.01 [d,A]	na
		2nd	5.08 ± 0.00 [a,A]	4.97 ± 0.02 [a,B]	8.63 ± 0.03 [b,B]	10.54 ± 0.07 [c,B]	4.92 ± 0.01 [ac,A]
		3rd	6.20 ± 0.01 [a,A]	4.58 ± 0.01 [a,C]	6.56 ± 0.03 [ab,C]	7.80 ± 0.05 [b,C]	4.92 ± 0.07 [a,A]
	PEEL	1st	4.39 ± 0.02 [a,A]	7.02 ± 0.10 [b,A]	2.97 ± 0.04 [c,A]	3.24 ± 0.01 [d,A]	na
		2nd	4.52 ± 0.03 [a,B]	6.89 ± 0.06 [b,A]	2.74 ± 0.05 [c,AB]	3.06 ± 0.10 [c,B]	4.60 ± 0.02 [a,A]
		3rd	6.09 ± 0.04 [ac,C]	7.01 ± 0.06 [b,A]	3.49 ± 0.01 [c,C]	2.71 ± 0.03 [d,C]	5.17 ± 0.01 [e,A]
	SEED	1st	4.55 ± 0.01 [a,A]	3.53 ± 0.02 [b,A]	2.48 ± 0.03 [c,A]	2.75 ± 0.03 [d,A]	na
		2nd	4.86 ± 0.01 [ac,B]	2.61 ± 0.02 [b,B]	4.00 ± 0.02 [a,B]	5.23 ± 0.04 [c,B]	3.68 ± 0.07 [ac,A]
		3rd	5.17 ± 0.06 [a,C]	2.53 ± 0.04 [b,C]	2.87 ± 0.04 [c,B]	5.51 ± 0.05 [d,C]	3.85 ± 0.04 [e,A]
Fiber	PULP	1st	1.04 ± 0.42 [a,A]	0.30 ± 0.03 [b,A]	0.31 ± 0.01 [b,A]	0.39 ± 0.06 [b,A]	na
		2nd	0.30 ± 0.04 [a,B]	0.30 ± 0.01 [a,A]	0.56 ± 0.04 [b,B]	0.61 ± 0.04 [b,A]	0.45 ± 0.03 [c,A]
		3rd	0.29 ± 0.06 [a,B]	0.32 ± 0.00 [a,A]	0.53 ± 0.12 [a,A]	0.92 ± 0.33 [a,A]	0.45 ± 0.04 [a,A]
	PEEL	1st	9.87 ± 0.37 [a,A]	16.28 ± 0.69 [b,A]	32.22 ± 0.14 [c,A]	34.74 ± 0.42 [d,A]	na
		2nd	23.56 ± 0.16 [a,B]	18.85 ± 0.10 [b,B]	35.88 ± 0.12 [c,B]	36.12 ± 0.37 [c,B]	29.84 ± 0.55 [d,A]
		3rd	22.30 ± 0.45 [a,B]	20.30 ± 0.11 [b,C]	30.33 ± 0.18 [c,A]	35.96 ± 0.10 [d,B]	27.94 ± 1.21 [e,A]
	SEED	1st	0.19 ± 0.00 [a,A]	0.20 ± 0.00 [a,A]	0.25 ± 0.01 [b,A]	0.20 ± 0.01 [a,A]	na
		2nd	0.22 ± 0.01 [a,B]	0.17 ± 0.01 [bc,B]	0.20 ± 0.00 [ab,B]	0.23 ± 0.02 [a,A]	0.16 ± 0.00 [c,A]
		3rd	0.22 ± 0.01 [a,B]	0.15 ± 0.00 [b,C]	0.17 ± 0.00 [c,C]	0.20 ± 0.00 [d,A]	0.15 ± 0.01 [b,B]
Ash	PULP	1st	6.86 ± 0.16 [a,A]	2.65 ± 0.02 [b,A]	2.77 ± 0.04 [b,A]	4.45 ± 0.07 [c,A]	na
		2nd	5.54 ± 0.11 [a,B]	3.10 ± 0.00 [b,B]	5.99 ± 0.02 [c,B]	6.92 ± 0.02 [d,B]	4.36 ± 0.02 [e,A]
		3rd	4.84 ± 0.24 [a,C]	2.65 ± 0.12 [b,A]	4.56 ± 0.06 [ac,C]	6.49 ± 0.12 [d,C]	4.37 ± 0.08 [c,A]
	PEEL	1st	3.42 ± 0.01 [a,A]	3.57 ± 0.00 [b,A]	2.66 ± 0.07 [c,A]	3.68 ± 0.03 [b,A]	na
		2nd	6.47 ± 0.13 [a,B]	2.69 ± 0.05 [b,B]	1.58 ± 0.04 [c,B]	2.65 ± 0.05 [b,B]	4.03 ± 0.03 [d,A]
		3rd	6.66 ± 0.14 [a,B]	2.56 ± 0.08 [b,B]	2.23 ± 0.04 [c,C]	2.91 ± 0.05 [d,C]	5.10 ± 0.07 [e,B]
	SEED	1st	3.08 ± 0.08 [a,A]	1.81 ± 0.02 [b,A]	1.94 ± 0.00 [b,A]	2.20 ± 0.01 [c,A]	na
		2nd	3.11 ± 0.09 [a,A]	1.62 ± 0.01 [b,B]	2.50 ± 0.06 [c,B]	2.99 ± 0.06 [a,B]	2.46 ± 0.03 [c,A]
		3rd	2.97 ± 0.07 [a,A]	1.92 ± 0.09 [b,A]	2.28 ± 0.08 [c,C]	2.71 ± 0.03 [d,C]	2.45 ± 0.04 [e,A]

Table 3 (*continued*)

PA	Tissue	Cycle	QG	RCF	RCG	CB	Hass
Sugars	PULP	1st	2.17 ± 0.06 a,A	0.72 ± 0.06 b,A	5.87 ± 0.20 c,A	2.88 ± 0.09 d,A	na
		2nd	1.74 ± 0.04 a,B	1.82 ± 0.04 a,B	9.20 ± 0.22 b,B	4.61 ± 0.07 c,B	1.39 ± 0.05 d,A
		3rd	2.65 ± 0.06 a,C	1.24 ± 0.03 b,C	5.71 ± 0.03 c,A	2.02 ± 0.03 d,C	1.24 ± 0.03 b,B
	PEEL	1st	2.90 ± 0.13 a,A	1.47 ± 0.10 b,A	2.88 ± 0.13 a,A	1.78 ± 0.03 b,A	na
		2nd	5.05 ± 0.32 a,B	3.51 ± 0.21 b,B	3.20 ± 0.13 b,A	2.25 ± 0.08 c,B	3.34 ± 0.14 b,A
		3rd	2.33 ± 0.05 a,C	5.99 ± 0.15 b,C	8.86 ± 0.08 c,B	1.10 ± 0.13 d,C	4.56 ± 0.14 e,B
	SEED	1st	2.30 ± 0.05 a,A	4.34 ± 0.10 b,A	6.04 ± 0.04 c,A	7.38 ± 0.16 d,A	na
		2nd	4.06 ± 0.05 a,B	2.75 ± 0.11 b,B	2.14 ± 0.03 c,B	3.28 ± 0.16 d,B	2.50 ± 0.08 b,A
		3rd	5.99 ± 0.18 a,C	2.31 ± 0.00 b,C	2.02 ± 0.05 b,C	2.87 ± 0.09 c,C	1.70 ± 0.03 b,B
Starch	SEED	1st	38.71 ± 1.02 ab,A	40.50 ± 1.46 ab,A	35.43 ± 2.09 a,A	41.26 ± 2.37 b,A	na
		2nd	24.23 ± 1.93 a,B	19.33 ± 0.72 b,B	21.38 ± 0.46 ab,B	19.75 ± 2.21 ab,B	36.95 ± 0.81 c,A
		3rd	29.40 ± 0.25 a,C	31.60 ± 0.73 abc,C	31.16 ± 1.33 ab,C	34.32 ± 2.04 bc,C	35.48 ± 1.49 c,A
Lipids	PULP	1st	55.78 ± 0.59 a,A	73.10 ± 1.39 a,A	63.32 ± 0.57 a,A	59.33 ± 1.17 a,A	na
		2nd	66.95 ± 0.66 a,B	70.89 ± 0.56 a,A	37.90 ± 0.94 b,B	36.82 ± 2.46 b,B	62.88 ± 4.75 a,A
		3rd	63.59 ± 0.08 ad,C	75.42 ± 1.02 b,A	58.73 ± 2.19 a,C	41.34 ± 1.04 c,B	69.84 ± 0.98 bd,A
	PEEL	1st	15.37 ± 1.72 a,A	27.65 ± 2.29 b,A	15.51 ± 2.93 c,A	7.51 ± 1.42 c,A	na
		2nd	5.02 ± 0.00 ab,B	7.85 ± 2.81 b,B	2.42 ± 0.27 a,B	1.88 ± 0.38 a,B	2.14 ± 0.27 a,A
		3rd	9.21 ± 1.23 a,C	12.76 ± 3.00 a,B	8.81 ± 0.15 b,B	6.17 ± 0.15 b,B	2.95 ± 0.27 bc,B
	SEED	1st	1.08 ± 0.27 a,A	4.32 ± 0.67 b,A	0.71 ± 0.15 c,A	0.88 ± 0.15 a,A	na
		2nd	8.19 ± 0.39 a,A	2.43 ± 0.23 a,B	2.03 ± 0.82 a,B	2.61 ± 0.29 a,B	1.41 ± 0.49 a,A
		3rd	2.77 ± 0.00 ab,A	2.00 ± 0.20 ab,B	0.83 ± 0.18 b,B	1.89 ± 0.16 b,C	4.12 ± 0.62 a,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 cycle); Values with different letter (a-d) along the row indicates significant statistical differences between varieties (Tukey HSD, p≤0.05). Different capital letter (A-B) along the column indicates significant statistical differences in same variety and tissue, in the distinct harvest cycles (years).; na- not analysed.

3.3.6. Pigments

3.3.6.1. Chlorophylls (a, b, and total)

Pigments are an important characteristic in avocado fruits, especially by an industrial and consumer point of view, since is the greatest indicator of fruit ripeness, as the change in color, even slight, is indicative of fruit ripening [170].

Relatively to chlorophylls and from the three parameters analyzed, could be state that the pulp was the tissue with higher content, followed by the peel and seed. In all the harvest cycles, the pulp values ranged from 0.92 to 7.43 mg/100g of chlorophyll *a*, while in chlorophyll *b* and total, the values spanned between 1.26 to 7.10 mg/100g and 2.31 to 13.83 mg/100g, respectively.

The by-products in the peel exhibited values ranging from 1.47 to 5.72 mg/100g, 0.65 to 2.96 mg/100g, and 2.19 to 8.93 mg/100g for chlorophylls *a*, *b*, and total, respectively. Meanwhile, in the seed values vary between 0.03 to 0.65 mg/100g in chlorophyll *a*, 0.11 to 1.89 mg/100g in chlorophyll *b*, and 0.14 to 2.64 mg/100g in total chlorophyll.

In a previous study performed in the peel of *Hass* avocado, it showed values for the three chlorophylls ranging from 3.1 to 4.3 mg/100g for chlorophyll *a*, 1.4 to 1.6 mg/100g for chlorophyll *b*, and 5.0 to 6.3 mg/100g in total chlorophyll, which was comparable to our findings, with slightly ascendent for our results [170]. Similar, Nguyen et al. [171] performed chlorophyll analysis in the pulp of fifteen avocado varieties, reaching values that oscillated between 13.8 to 31.6 mg/100g for chlorophyll *a*, and 4.7 to 22.4 mg/100g for chlorophyll *b*, which were a little bit higher than our findings. Other study performed the total chlorophyll analysis in the pulp, peel and seed of three commercial varieties, *Margarida*, *Geada*, and *Breda*, displaying values much higher than our results, with values ranging between 538 to 808 mg/100g in the pulp, 943 to 1101 mg/100g in the peel, and 150 to 634 mg/100g in the seed [172].

Significant differences ($p \leq 0.05$) between the varieties in the different harvest cycles were examined, it was visible the high number of differences in these parameters (Table 4). In the first harvest cycle, it was observed for the pulp that all the varieties displayed differences in the chlorophyll *a* parameter, while in chlorophyll *b*, only the QG variety showed a difference relatively to the others. In total chlorophylls, QG variety displayed differences to all varieties. In the second harvest cycle, the pulp showed a similarity between RCG and CB varieties, with the *Hass* variety differing from all in the chlorophyll *a* and *b* parameters. In total chlorophyll, all varieties demonstrated a similar

pattern as the previous chlorophyll parameters, with the additional of CB variety exhibiting similarity to both RCF and QG varieties. In the final harvest cycle of the pulp, in chlorophyll *a*, only the QG and CB varieties exhibited similarity. In chlorophyll *b*, all varieties showed differences, with exception of CB and *Hass* varieties that resembled between them. Conversely, in total chlorophylls, only the RCG variety exhibited similarity to the CB variety.

Moving on to the first harvest cycle of the peel, the RCF and RCG varieties demonstrated similarity in chlorophyll *a* content, while both QG and RCF showed similarity in chlorophyll *b*, as well as between RCG and CB. In total chlorophyll, the RCG and CB varieties resembled, while all the other differed. In the second harvest cycle, the QG variety showed similarity to both RCF and *Hass* varieties for chlorophyll *a*, while the QG, RCG, and *Hass* varieties exhibited similarity in chlorophyll *b*. All varieties displayed differences in total chlorophyll, except for QG and *Hass* that showed resemble together. In the last harvest cycle, CB and *Hass* differed from each other, in both chlorophyll *a* and total while the other varieties showed resemblance to themselves, and to the priors ones. RCF and *Hass* showed resemblance in chlorophyll *b*, while CB differed from them. The QG and RCG varieties displayed similarities between themselves and resembled all varieties.

Going to the first harvest cycle of the seed, in all chlorophylls, the same pattern was followed, with the QG variety displaying differences from all the other varieties. In the second harvest cycle, it was not detected any differences between the varieties. The third harvest cycle revealed a similarity between the QG and *Hass* varieties, while the RCF variety differed from all. The RCG variety resembles to QG, CB, and *Hass* varieties, meanwhile, the CB variety displays similarity with the RCF and RCG varieties.

In table 4 significant statistical differences ($p \leq 0.05$) were observed through the harvest cycles and from each variety and tissues, following the capital letters by column.

In the pulp, chlorophylls *a* and total achieved the same statistical distribution, where all the regionals varieties shown differences in all the harvest cycles, while the commercial variety *Hass* showed no differences in its harvest cycles. The QG and RCF varieties exhibited distinctions in chlorophyll *b,* in the initial harvest cycle compared to the other two cycles, whereas the CB variety manifested variations in the second harvest cycle. In contrast, the RCG variety displayed differences across all harvest cycles, while the *Hass* variety does not show distinctions.

The QG and RCF varieties showed no differences in chlorophyll *a,* in the peel, across cycles, while the RCG and *Hass* varieties showed only differences in all cycles. In the CB variety, only the third harvest cycle showed differences in relation to other cycles. The QG variety showed similarities in chlorophyll *b*, contrarily to only differences observed in the

Hass variety. The other three varieties showed one harvest cycle diverging from the others, which in the RCF variety was the third harvest cycle, while in the RCG and CB varieties the second harvest cycle was the outsider. The RCF variety exhibited a notable difference in total chlorophyll content, in the third harvest cycle compared to the other cycles, whereas in the CB variety, the distinctions were observed between the second and third harvest cycles, with the first cycle resembling both. In contrast, the RCG and *Hass* varieties displayed differences across all cycles, while the QG variety showed no discernible distinctions.

In the seed, across the harvest cycles, all regional varieties exhibited the same pattern in all the chlorophyll parameters, in which only the QG variety displayed differences, with the first harvest cycle being different from the others. Meanwhile, in the commercial variety *Hass*, the similarity showed in chlorophyll *a*, was replaced by differences across the harvest cycles in both chlorophyll *b* and total.

3.3.6.2. Carotenoids

Carotenoids are a huge source of antioxidant that are related to many processes in living organisms, and naturally the analysis of this parameter was required as avocado fruit contains a good amount of this compound [16]. In this parameter, several carotenoids were analyzed, being the most important ones in avocado, the lutein and the zeaxanthin.

Our avocado varieties showed values of β-carotene, in dry weight, ranging from 0.85 to 4.73 mg/100g in the pulp, while in by-products values stayed between 0.11 to 3.74 mg/100g and 0.04 to 2.59 mg/100g in the peel and seed, respectively. There was a general decrease in content of this parameter from the second to the third harvest cycle.

Moving to carotenoid lutein, our values oscillated between 1.11 to 4.93 mg/100g in the pulp, 0.76 to 3.04 mg/100g in the peel, and lastly 0.37 to 2.26 mg/100g in the seed.

The analysis of the carotenoid lycopene revealed values that ranged between 1.01 to 5.43 mg/100g in the pulp, with the lowest content present in the commercial variety *Hass*. In the by-products, values displayed spanning from 0.13 to 4.31 mg/100g in the peel and 0.01 to 3.12 mg/100g in the seed.

The zeaxanthin content in avocado ranges between 0.95 to 5.29 mg/100g in the pulp, with the *Hass* variety being the poorest in this carotenoid content, while the peel and seed obtained values of 0.13 to 4.19 mg/100g and 0.04 to 2.90 mg/100g, respectively.

Two different extractions were conducted using distinct solvents to analyze the total carotenoid content in our varieties. One analysis utilized ethanol (EtOH), while the other employed acetone (Ac). The total carotenoid (EtOH), it was detected values in the pulp

oscillating between 0.68 to 2.63 mg/100g, while in the peel between 0.50 to 2.98 mg/100g, and 0.23 to 2.62 mg/100g in the seed was obtained.

The values of total carotenoids (Ac) stayed between 0.92 to 4.94 mg/100g in the pulp, while in the by-products, the values ranged between 0.15 to 3.92 mg/100g in the peel and 0.01 to 2.82 mg/100g in the seed.

Overall, the total carotenoid content (Ac) exhibited consistently higher levels across all tissues and harvest cycles, than the total carotenoids (EtOH). It is noteworthy that in various carotenoid analyses, the commercial *Hass* variety consistently demonstrated the lowest content, particularly in the pulp, but also in the other tissue analyses.

Numerous research have been conducted carotenoid analyses in avocados. For instance, a study by Vinha et al. [39] analyzed carotenoids in the *Hass* avocado variety, cultivated in the Algarve, revealing values of 0.82 mg/100g fresh weight (FW) 2.59 mg/100g FW, and 0.97 mg/100g FW in the pulp, peel, and seed, respectively, which were a little bit lower than our findings, but expected due to work with the fresh fruit. Similar, Hong et al. [173] determined the individual and total carotenoid concentration content in five avocado cultivars, where the values obtained range between 1.12 to 2.22 mg/100g DW for lutein, 0.03 to 0.06 mg/100g DW for zeaxanthin, 0.07 to 0.16 mg/100g DW for β-carotene, and 3.33 to 7.26 mg/100g DW for total carotenoid content. From all this values, lutein was slightly lower than our findings, both zeaxanthin and β-carotene revealed values about 10 to 100 times lower than our findings, less than our results. In contrast to total carotenoids, our findings stayed a little bit behind its values.

Another research executed by Ramos-Aguilar et al. [174] analyzed the individual carotenoids presented in the pulp and peel of two avocado varieties, and obtained values oscillating between 0.49 to 1.20 mg/100g DW in the pulp and 4.54 to 10.38 mg/100g DW in the peel for lutein. The zeaxanthin ranged from 0.02 to 0.03 mg/100g DW and 0.10 to 0.32 mg/100g DW for the pulp and peel, respectively. The β-carotene only was detected in the peel, with values spanning between 0.22 to 0.64 mg/100g DW. In comparison, the lutein levels were like ours in the pulp, but higher in the peel level. Like the previous study, zeaxanthin stayed much lower than our findings, and β-carotene level were a bit higher than our results.

These carotenoids analysis was very important to have a real perception of the it content in the avocados harvested in Madeira Island. We can see that Madeira avocado varieties are among the richest in carotenoids, which is very significant, since they play a notorious role in deactivate free radicals in human cells [175]. Also, to help carotenoids

performing its functions properly, products with high oil content, like avocado, facilitates the absorption of the carotenoids [59].

The small letter in Table 4 shows significant differences ($p \leq 0.05$) in carotenoids, between the varieties in the different harvest cycles.

In the pulp, there is no detected difference between varieties in the β-carotene parameter, in the first harvest cycle, while in lutein and total carotenoids (EtOH), the same pattern was followed, with the QG variety displaying differences relatively to the others. Similarly, zeaxanthin and total carotenoids (Ac) exhibited similarities between the RCF and CB varieties, and differences with the RCG variety, while the QG variety showed resemble to all. In lycopene, the RCF and RCG demonstrated differences, while QG and CB showed similarities between themselves and all the other varieties.

The four carotenoids, β-carotene, lycopene, zeaxanthin, and total carotenoids (Ac) in the pulp, second harvest cycle, exhibited the same behavior, with most varieties showing differences, except for the QG and RCG varieties that show to be similar. Similarly, the total carotenoids (EtOH) of RCF and CB varieties are similar, while all varieties demonstrated differences in lutein.

In the third harvest cycle of the pulp, in β-carotene, the RCG and *Hass* varieties shows resemblance, while the other varieties resembled themselves and all the varieties. Lycopene and total carotenoids (Ac) followed the same path, with two groups formed, one by the QG, RCG, and CB varieties, while the other formed by the RCF and *Hass* varieties, based on its similarities. Meanwhile, the total carotenoids (EtOH) displayed all differences between all varieties, while the same happened in the lutein, except for the QG and RCG varieties, that were similar. The QG and CB varieties differed in zeaxanthin content from all, while the RCF and *Hass* varieties showed resemblance. The RCG variety demonstrated similarity to QG and CB varieties.

In the first harvest cycle of the peel, there was no detected difference between the varieties in the carotenoids, except for the lutein that displayed a difference between all the varieties, excluding the RCG and CB varieties that showed resemblance. In the second harvest cycle, the variations between varieties were greater, where the four carotenoids parameters, β-carotene, lycopene, zeaxanthin and total carotenoids (Ac) have the same pattern, with the RCF and CB varieties showing differences, while the RCG and *Hass* varieties resembled themselves and with CB variety. The QG variety showed similarities with the RCF, RCG and *Hass* varieties. In terms of total carotenoids (EtOH), the QG and RCF varieties showed similarities. All the others shown differences. On the other hand, in lutein, given the differences between the varieties presented, only the *Hass* variety showed

similarity with the QG and RCF varieties. In the final stage of peeling, three carotenoids, β-carotene, zeaxanthin, and total carotenoids (Ac) followed a similar pattern. The RCF and CB types exhibited distinctions, whereas the QG and RCG varieties mirrored each other and the CB type. The *Hass* variety demonstrated similarity with the QG, RCF, and RCG varieties. Two groups were formed in lutein, with the QG and RCG resembling, as the same happened between the RCF and *Hass* varieties. The CB variety differed from all, except the QG and RCG varieties. In lycopene, like in previous carotenoids parameter, the RCF and CB exhibited distinctions, whereas the QG and RCG varieties mirrored each other and the CB variety. The *Hass* variety demonstrated similarity with the QG, RCF, and RCG varieties, and the QG variety showed similarities to all varieties. Lastly, in total carotenoids (EtOH), the CB and *Hass* varieties differed, while the RCG variety resembled to all the other varieties. The QG variety showed similarities to the RCF, RCG, and CB varieties, meanwhile the RCF variety mirrored the QG, RCG, and *Hass* varieties.

Almost every carotenoid of seed in the first harvest cycle showed the same path, with the QG variety differing from the other varieties, only in total carotenoids (EtOH). In addition to what happened previously, the RCG variety demonstrated similarity with all the varieties. In the second harvest cycle of the seed, β-carotene, zeaxanthin, and total carotenoids (Ac) followed a similar trajectory with no differences detected, while in total carotenoids (EtOH), all varieties were different. In lutein, apart all differences between regionals varieties, *Hass* variety showed similarities with RCG and CB varieties. In lycopene, the CB variety differed from all varieties, meanwhile, the RCF and RCG were similar. The QG variety was close to the CB and *Hass* varieties, while *Hass* variety mirrored the QG, RCF, and RCG varieties. The lutein and total carotenoids (EtOH), in the last cycle, showed differences between varieties, while the other carotenoids followed the same pattern with, the QG and CB varieties displayed differences to all varieties, while the RCG and *Hass* varieties resembled. The RCF variety mirrored the RCG, CB, and *Hass* varieties.

In the Table 4 (following capital letters) significant statistical differences ($p \leq 0.05$) where observed inside the varieties through the harvest cycles and tissues.

Related to the carotenoids in the pulp of the QG variety, a resemblance between β-carotene and lutein was observed, as no differences were detected in the different harvest cycles, unlike what happened with total carotenoids (EtOH), where all the cycles revealed differences between them. Lycopene and total carotenoids (Ac) displayed the same path in which the second and third harvest cycles differed, and the first harvest cycle presented similarities to both, while in zeaxanthin, only the third harvest cycle exhibited differences among the others.

In the peel, differences in the carotenoids between the harvest cycles in each variety were not detected. Meanwhile, in the seed, differences were achieved, as differences in β-carotene, lycopene, zeaxanthin, and total carotenoids (Ac), followed by a difference in the first harvest cycle related to others in lutein and total carotenoids (EtOH).

The pulp of the RCF variety mimicked the same disposition found in the seed of the QG variety, with most carotenoids displaying differences in all harvest cycles, while other carotenoids presented a variation in the first harvest cycle. In the peel, while the carotenoids, β-carotene, lycopene, zeaxanthin, and total carotenoids (Ac) did not show any differences over the cycles, the total carotenoids (EtOH) only showed differences. In lutein, differences between the first and second harvest cycles were detected. In the seed, no differences were observed between the harvest cycles in β-carotene, zeaxanthin, and total carotenoids (Ac), while lutein and total carotenoids (EtOH) showed variations across all the cycles. Lycopene exhibited differences in the first and third harvest cycles, whereas the second cycle resembled them.

Moving on to the RCG variety, in the pulp, most carotenoids (β-carotene, lycopene, zeaxanthin, and total carotenoids (Ac)) followed the same path, with the third harvest cycle showing distinction to the others, while in total carotenoids (EtOH) it was the first cycle that presented differences to the others. All the differences in the harvest cycles were achieved in the lutein. In the peel and seed, β-carotene, lycopene, zeaxanthin, and total carotenoids (Ac) demonstrated no differences between the harvest cycles while, meanwhile the lutein and carotenoids (EtOH) exhibited differences between the cycles.

Going on to CB variety, in the pulp, all the carotenoids presented differences between the harvest cycles, except for the total carotenoid (Ac), that exhibited differences between the first and the other two cycles. In the peel, β-carotene, zeaxanthin, and total carotenoids (Ac) followed the same pattern, with the second and third harvest cycles displaying differences, while the first one mirrored both. No differences were detected in lycopene and carotenoids (EtOH), while in lutein only the third harvest cycle stranded out from the others. In relation to the seed, all varieties displayed a consistent distribution, with the second harvest cycle differing from the others. The exception was lutein, where the first harvest cycle showed variation compared to the others.

Advancing to the *Hass* variety, in the pulp, all varieties showed differences in all the harvest cycles, except in lutein, that showed similarities in both cycles. In the by-products, all the carotenoids presented differences between cycles.

Table 4 Chlorophylls (a, b, and total) and carotenoids (β-carotene, lutein, lycopene, zeaxanthin, and total) evaluation in five different avocado varieties.

			Varieties				
PA	Tissue	Cycle	QG	RCF	RCG	CB	Hass
Chlorophyll a	PULP	1st	3.92 ± 0.36 ^{a,A}	0.97 ± 0.08 ^{b,A}	1.52 ± 0.13 ^{c,A}	1.64 ± 0.11 ^{c,A}	na
		2nd	7.43 ± 0.18 ^{a,B}	2.68 ± 0.08 ^{b,B}	6.27 ± 0.07 ^{c,B}	6.02 ± 0.31 ^{c,B}	0.92 ± 0.05 ^{d,A}
		3rd	4.88 ± 0.15 ^{a,C}	1.86 ± 0.07 ^{b,C}	3.38 ± 0.10 ^{c,C}	4.88 ± 0.20 ^{a,C}	0.98 ± 0.13 ^{d,A}
	PEEL	1st	5.72 ± 0.32 ^{a,A}	4.70 ± 0.32 ^{b,A}	2.16 ± 0.05 ^{b,A}	1.82 ± 0.14 ^{c,A}	na
		2nd	4.14 ± 0.30 ^{ad,A}	4.44 ± 0.05 ^{a,A}	2.83 ± 0.07 ^{b,B}	1.79 ± 0.04 ^{c,A}	3.80 ± 0.16 ^{d,A}
		3rd	4.78 ± 0.05 ^{ab,A}	3.89 ± 0.11 ^{ab,A}	2.49 ± 0.02 ^{ab,C}	1.47 ± 0.07 ^{a,B}	4.97 ± 0.11 ^{b,B}
	SEED	1st	0.65 ± 0.26 ^{a,A}	0.13 ± 0.11 ^{b,A}	0.10 ± 0.09 ^{b,A}	0.13 ± 0.12 ^{b,A}	na
		2nd	0.06 ± 0.01 ^{a,B}	0.19 ± 0.11 ^{a,A}	0.31 ± 0.01 ^{a,A}	0.09 ± 0.06 ^{a,A}	0.05 ± 0.01 ^{a,A}
		3rd	0.03 ± 0.00 ^{a,B}	0.15 ± 0.02 ^{b,A}	0.07 ± 0.01 ^{ac,A}	0.12 ± 0.01 ^{bc,A}	0.04 ± 0.00 ^{a,A}
Chlorophyll b	PULP	1st	3.39 ± 0.17 ^{a,A}	1.26 ± 0.20 ^{b,A}	1.56 ± 0.19 ^{b,A}	1.26 ± 0.10 ^{b,A}	na
		2nd	4.71 ± 0.19 ^{a,B}	2.98 ± 0.15 ^{b,B}	7.10 ± 0.19 ^{c,B}	6.50 ± 0.54 ^{c,B}	1.48 ± 0.01 ^{d,A}
		3rd	4.61 ± 0.13 ^{a,B}	2.77 ± 0.18 ^{b,B}	3.61 ± 0.10 ^{c,C}	1.72 ± 0.06 ^{d,A}	1.53 ± 0.12 ^{d,A}
	PEEL	1st	2.93 ± 0.16 ^{a,A}	2.96 ± 0.15 ^{a,A}	1.00 ± 0.07 ^{b,A}	0.69 ± 0.10 ^{b,A}	na
		2nd	1.77 ± 0.14 ^{a,A}	2.93 ± 0.05 ^{b,A}	1.58 ± 0.07 ^{a,B}	1.01 ± 0.04 ^{c,B}	1.74 ± 0.10 ^{a,A}
		3rd	2.03 ± 0.14 ^{ab,A}	2.36 ± 0.08 ^{a,B}	1.42 ± 0.06 ^{ab,B}	0.65 ± 0.08 ^{b,A}	2.43 ± 0.09 ^{a,B}
	SEED	1st	1.89 ± 0.85 ^{a,A}	0.37 ± 0.32 ^{b,A}	0.25 ± 0.24 ^{b,A}	0.39 ± 0.30 ^{b,A}	na
		2nd	0.23 ± 0.02 ^{a,B}	0.45 ± 0.00 ^{a,A}	0.88 ± 0.04 ^{a,A}	0.44 ± 0.14 ^{a,A}	0.25 ± 0.02 ^{a,A}
		3rd	0.11 ± 0.06 ^{a,B}	0.46 ± 0.01 ^{b,A}	0.26 ± 0.02 ^{c,A}	0.40 ± 0.03 ^{b,A}	0.19 ± 0.00 ^{ac,B}
Chlorophyll total	PULP	1st	7.56 ± 0.52 ^{a,A}	2.31 ± 0.30 ^{b,A}	3.19 ± 0.33 ^{b,A}	2.99 ± 0.22 ^{b,A}	na
		2nd	12.53 ± 0.38 ^{a,B}	5.86 ± 0.24 ^{b,B}	13.83 ± 0.27 ^{c,B}	12.94 ± 0.88 ^{ac,B}	2.48 ± 0.06 ^{d,A}
		3rd	9.81 ± 0.28 ^{a,C}	4.79 ± 0.26 ^{b,C}	7.24 ± 0.21 ^{c,C}	7.06 ± 0.67 ^{c,C}	2.61 ± 0.26 ^{d,A}
	PEEL	1st	8.93 ± 0.50 ^{a,A}	7.90 ± 0.49 ^{b,A}	3.26 ± 0.12 ^{c,A}	2.58 ± 0.24 ^{c,AB}	na
		2nd	6.10 ± 0.45 ^{a,A}	7.61 ± 0.04 ^{b,A}	4.54 ± 0.11 ^{c,B}	2.89 ± 0.08 ^{d,A}	5.71 ± 0.26 ^{a,A}
		3rd	7.02 ± 0.20 ^{ab,A}	6.45 ± 0.19 ^{ab,B}	4.03 ± 0.06 ^{ab,C}	2.19 ± 0.15 ^{a,B}	7.63 ± 0.11 ^{b,B}
	SEED	1st	2.64 ± 1.15 ^{a,A}	0.52 ± 0.45 ^{b,A}	0.36 ± 0.34 ^{b,A}	0.54 ± 0.44 ^{b,A}	na
		2nd	0.30 ± 0.03 ^{a,B}	0.60 ± 0.06 ^{a,A}	1.24 ± 0.03 ^{a,A}	0.46 ± 0.22 ^{a,A}	0.31 ± 0.03 ^{a,A}
		3rd	0.14 ± 0.06 ^{a,B}	0.64 ± 0.01 ^{b,A}	0.34 ± 0.03 ^{c,A}	0.52 ± 0.05 ^{b,A}	0.24 ± 0.00 ^{ac,B}

Table 4 (*continued*)

PA	Tissue	Cycle	QG	RCF	RCG	CB	Hass
β-carotene	PULP	1st	4.39 ± 0.37 [a,A]	3.66 ± 0.09 [a,A]	4.73 ± 0.58 [a,A]	3.63 ± 0.16 [a,A]	na
		2nd	4.47 ± 0.27 [a,A]	1.87 ± 0.05 [b,B]	4.39 ± 0.07 [a,A]	2.59 ± 0.10 [c,B]	1.08 ± 0.06 [d,A]
		3rd	2.77 ± 0.13 [ab,A]	1.16 ± 0.10 [ab,C]	2.82 ± 0.11 [b,B]	2.30 ± 0.08 [ab,C]	0.85 ± 0.13 [b,B]
	PEEL	1st	3.74 ± 0.01 [a,A]	3.16 ± 0.05 [a,A]	0.84 ± 0.08 [a,A]	1.36 ± 0.08 [a,AB]	na
		2nd	2.93 ± 0.21 [ab,A]	3.38 ± 0.20 [a,A]	2.41 ± 0.07 [bc,A]	1.32 ± 0.07 [c,A]	1.81 ± 0.09 [bc,A]
		3rd	2.60 ± 0.52 [ac,A]	3.70 ± 0.09 [b,A]	1.11 ± 0.17 [ac,A]	0.11 ± 0.04 [a,B]	2.60 ± 0.13 [bc,B]
	SEED	1st	1.89 ±0.28 [a,A]	0.00 ± 0.00 [b,A]	0.00 ± 0.01 [b,A]	0.04 ± 0.06 [b,A]	na
		2nd	1.14 ± 0.15 [a,B]	2.59 ± 4.26 [a,A]	0.04 ± 0.09 [a,A]	1.38 ± 0.18 [a,B]	0.27 ± 0.00 [a,A]
		3rd	0.50 ± 0.14 [a,C]	0.16 ± 0.01 [bc,A]	0.00 ± 0.10 [b,A]	0.25 ± 0.00 [c,A]	0.00 ± 0.07 [b,B]
Luteín	PULP	1st	2.80 ± 0.19 [a,A]	1.11 ± 0.07 [b,A]	1.29 ± 0.12 [b,A]	1.14 ± 0.10 [b,A]	na
		2nd	3.85 ± 0.10 [a,A]	2.55 ± 0.07 [b,B]	4.93 ± 0.07 [c,B]	4.16 ± 0.15 [d,B]	1.65 ± 0.02 [e,A]
		3rd	3.99 ± 0.09 [a,A]	2.59 ± 0.12 [b,B]	3.89 ± 0.08 [a,C]	3.40 ± 0.12 [c,C]	1.77 ± 0.11 [d,A]
	PEEL	1st	3.04 ± 0.21 [a,A]	2.56 ± 0.16 [b,A]	1.11 ± 0.05 [c,A]	0.92 ± 0.09 [c,A]	na
		2nd	2.02 ± 0.16 [a,A]	2.27 ± 0.02 [b,B]	1.42 ± 0.02 [c,B]	0.96 ± 0.02 [d,A]	2.13 ± 0.11 [ab,A]
		3rd	2.07 ± 0.04 [ab,A]	2.40 ± 0.07 [b,AB]	1.63 ± 0.01 [ab,C]	0.76 ± 0.04 [a,B]	2.68 ± 0.06 [b,B]
	SEED	1st	1.02 ± 0.14 [a,A]	0.37 ± 0.09 [b,A]	0.48 ± 0.07 [b,A]	0.41 ± 0.10 [b,A]	na
		2nd	0.45 ± 0.04 [a,B]	2.26 ± 0.09 [b,B]	1.60 ± 0.05 [c,B]	1.74 ± 0.02 [d,B]	1.67 ± 0.01 [cd,A]
		3rd	0.39 ± 0.03 [a,B]	0.78 ± 0.01 [b,C]	0.61 ± 0.01 [c,C]	1.88 ± 0.07 [d,B]	1.53 ± 0.00 [e,B]
Licopene	PULP	1st	4.76 ± 0.54 [ab,AB]	4.20 ± 0.11 [b,A]	5.43 ± 0.67 [a,A]	4.29 ± 0.17 [ab,A]	na
		2nd	5.35 ± 0.32 [a,A]	2.27 ± 0.06 [b,B]	5.23 ± 0.08 [a,A]	3.19 ± 0.12 [c,B]	1.28 ± 0.06 [d,A]
		3rd	3.21 ± 0.15 [a,B]	1.34 ± 0.11 [b,C]	3.33 ± 0.13 [a,B]	2.81 ± 0.09 [a,C]	1.01 ± 0.15 [b,B]
	PEEL	1st	4.31 ± 0.02 [a,A]	3.52 ± 0.05 [a,A]	0.90 ± 0.11 [a,A]	1.61 ± 0.10 [a,A]	na
		2nd	3.46 ± 0.25 [ab,A]	3.83 ± 0.24 [a,A]	2.72 ± 0.07 [bc,A]	1.51 ± 0.08 [c,A]	2.10 ± 0.10 [bc,A]
		3rd	3.04 ± 0.61 [abc,A]	4.22 ± 0.10 [b,A]	1.27 ± 0.19 [ac,A]	0.13 ± 0.04 [a,A]	3.01 ± 0.15 [bc,B]
	SEED	1st	2.29 ± 0.33 [a,A]	nd	0.05 ± 0.03 [b,A]	0.17 ± 0.10 [b,A]	na
		2nd	1.39 ± 0.18 [ac,B]	3.12 ± 0.09 [b,AB]	0.07 ± 0.02 [b,A]	1.74 ± 0.21 [c,B]	0.33 ± 0.00 [ab]
		3rd	0.62 ± 0.17 [a,C]	0.20 ± 0.01 [bc,B]	nd	0.30 ± 0.01 [c,A]	nd

Table 4 (*continued*)

PA	Tissue	Cycle	QG	RCF	RCG	CB	Hass
Xeaxanthin	PULP	1st	4.67 ± 0.52 ab,A	4.10 ± 0.11 b,A	5.29 ± 0.65 a,A	4.06 ± 0.17 b,A	na
		2nd	5.01 ± 0.31 a,A	2.09 ± 0.05 b,B	4.92 ± 0.08 a,A	2.90 ± 0.11 c,B	1.21 ± 0.06 d,A
		3rd	3.42 ± 0.56 a,B	1.30 ± 0.11 b,C	3.15 ± 0.13 ac,B	2.57 ± 0.09 c,C	0.95 ± 0.15 b,B
	PEEL	1st	4.19 ± 0.02 a,A	3.53 ± 0.06 a,A	0.91 ± 0.10 a,A	1.53 ± 0.09 a,AB	na
		2nd	3.29 ± 0.24 ab,A	3.79 ± 0.23 a,A	2.70 ± 0.07 bc,A	1.48 ± 0.07 c,A	2.03 ± 0.10 bc,A
		3rd	2.91 ± 0.59 ac,A	4.15 ± 0.10 b,A	1.24 ± 0.19 ac,A	0.13 ± 0.04 a,B	2.91 ± 0.14 bc,B
	SEED	1st	2.11 ± 0.31 a,A	nd	nd	0.04 ± 0.07 b,A	na
		2nd	1.28 ± 0.17 a,B	2.90 ± 0.09 a,A	0.05 ± 0.10 a	1.55 ± 0.20 a,B	0.31 ± 0.00 a,A
		3rd	0.56 ± 0.16 a,C	0.18 ± 0.01 bc,A	nd	0.28 ± 0.00 c,A	0.00 ± 0.00 b,B
Total Carotenoids (EtOH)	PULP	1st	1.61 ± 0.10 a,A	0.72 ± 0.05 b,A	0.85 ± 0.05 b,A	0.68 ± 0.06 b,A	na
		2nd	1.92 ± 0.03 a,B	1.48 ± 0.02 b,B	2.40 ± 0.04 c,B	1.53 ± 0.06 b,B	1.04 ± 0.02 d,A
		3rd	2.63 ± 0.05 a,C	1.55 ± 0.06 b,B	2.36 ± 0.05 c,B	1.93 ± 0.08 d,C	1.21 ± 0.08 e,B
	PEEL	1st	2.04 ± 0.08 a,A	2.07 ± 0.08 a,A	2.98 ± 0.04 a,A	2.38 ± 1.53 a,A	na
		2nd	1.29 ± 0.11 a,A	1.32 ± 0.03 a,B	0.87 ± 0.03 b,B	0.60 ± 0.00 c,A	1.58 ± 0.08 d,A
		3rd	1.38 ± 0.02 ab,A	1.71 ± 0.05 bc,C	1.16 ± 0.01 abc,C	0.50 ± 0.01 a,A	1.90 ± 0.07 c,B
	SEED	1st	0.64 ± 0.16 a,A	0.23 ± 0.04 b,A	0.43 ± 0.02 ab,A	0.27 ± 0.02 b,A	na
		2nd	0.40 ± 0.02 a,B	2.62 ± 0.04 b,B	1.39 ± 0.09 c,B	1.83 ± 0.05 d,B	1.61 ± 0.01 e,A
		3rd	0.37 ± 0.03 a,B	0.83 ± 0.01 b,C	0.59 ± 0.01 c,C	1.70 ± 0.06 d,A	1.59 ± 0.00 e,B
Total Carotenoids (Ac)	PULP	1st	4.34 ± 0.49 ab,AB	3.82 ± 0.10 b,A	4.94 ± 0.61 a,A	3.87 ± 0.16 b,A	na
		2nd	4.81 ± 0.29 a,A	2.03 ± 0.05 b,B	4.73 ± 0.07 a,A	2.85 ± 0.11 c,B	1.16 ± 0.06 d,A
		3rd	2.92 ± 0.14 a,B	1.22 ± 0.10 b,C	3.03 ± 0.12 a,B	2.54 ± 0.09 a,B	0.92 ± 0.14 b,B
	PEEL	1st	3.92 ± 0.01 a,A	3.23 ± 0.05 a,A	0.86 ± 0.09 a,A	1.46 ± 0.09 a,AB	na
		2nd	3.13 ± 0.22 ab,A	3.51 ± 0.21 a,A	2.50 ± 0.07 bc,A	1.38 ± 0.07 c,A	1.91 ± 0.09 bc,A
		3rd	2.76 ± 0.55 ac,A	3.86 ± 0.10 b,A	1.16 ± 0.18 ac,A	0.15 ± 0.06 a,B	2.74 ± 0.14 bc,B
	SEED	1st	2.06 ± 0.30 a,A	nd	0.01 ± 0.02 b	0.05 ± 0.08 b,A	na
		2nd	1.25 ± 0.16 a,B	2.82 ± 0.08 a,A	nd	1.56 ± 0.19 a,B	0.30 ± 0.00 a
		3rd	0.47 ± 0.08 a,C	0.18 ± 0.01 bc,A	nd	0.27 ± 0.01 c,A	nd

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≤0.05). Different capital letter (A-B) along the column indicates significant statistical differences in same variety and pigment parameter, in the distinct harvest cycles (years).; nd- not detected.

3.4. PCA of proximate analysis

One great statistical way to observe our varieties dispersion into the several parameters in study is the PCA analysis, which combines all information obtained in the variables to the subject (cases). Four different graphics (Fig. A1) were obtained, one for each tissue and one with all the tissues.

The PCA of the proximate analysis, of all tissues showed a good separation between the tissues. The first two principal components explain 69.86% of variance. First component 1 explained 43.40% of variance, with eigenvalues of 11.71, while component 2 explained 26.46% with eigenvalues of 7.14. As already expected, the major component was associated to each tissue. In all pulp varieties, the parameter highly associated was the lipid content, while in all the peel varieties, the crude fiber was the parameter highly associated. Since starch was only found in the seed, naturally was closely associate with this parameter.

Regarding the PCA per tissue, it was observed a high spatial difference between the regional and commercial ones in the pulp. The first two principal components accumulated 93.26% of variance. Component 1 explained 83.53% of variance, with eigenvalues of 8.52, while component 2 explained 9.73% with eigenvalues of 0.99. The regional varieties were in the direction of the main variable vectors, while the commercial one was separated from that grouping, being located in the opposed direction, and consequently revealing the lowest content in the parameters analyzed. Also, inside the regional varieties, it is possible to observe that the QG and RCF varieties resembled themselves, and the RCG with CB varieties.

In the PCA for the peel, the first two principal components accumulated 92.69% of variance. Component 1 explained 74.46% of variance, with eigenvalues of 7.96, while component 2 explained 18.23% with eigenvalues of 1.95. It was detected in the peel varieties shown almost the same described distribution that was found in the pulp, with the commercial variety being highly separated from the regional ones, and the same grouping, according to parameter similarities shared between the QG and RCF, and with the RCG and CB.

In the PCA for the seed, the first two principal components accumulated 96.48% of variance. Component 1 explained 87.10% of variance, with eigenvalues of 8.69, while component 2 explained 9.38% with eigenvalues of 0.94. It was observed a greater dispersion of the main vectors, where the regional varieties were dispersed along the vectors, and the tendency repeated for the commercial variety, by remaining in the opposite way of the main vectors and of the regional varieties discrimination.

3.5. Interaction between proximate parameters

The nutritional parameters are very important in foods, especially in fruits, since they change according to the fruit maturity stage, which is often judge by both external (color, texture, size) and internal attributes (acidity, starch, sugars) [176].

The variability found in our analysis was influenced by several factors, including in pre-harvest and postharvest, but there is no doubt that the maturity stage at harvest was possible the most determining factor in the final product quality[176].

This was visible in our analysis were all cycles revealed variability, in which ripeness at the time of harvest that dictated the composition of the fruit.

The pH and titratable acidity are important parameters in fruits and vegetables, closely linked as TA decreases during various metabolic processes, leading to higher pH values due to reduced acidity [147]. In our findings the pH values increased in our more mature harvest cycle (3rd), while TA values also increased contrary to expectations. This could be associated to the ripening process, since that, in this harvest cycle the ripening was not done entirely on the bench, but also in the refrigerator which is known for having a positive reaction on TA values [147].

The intake of crude fiber is vital in human diet since its consumption has been closely associated to the decreased risk of several diseases and maintenance of laxation system [177]. As noted before, in avocado crude fiber is strongly related to the peel and its notable that in our third harvest cycle (one-tree maturation) almost every variety decreased its values. This probably is related to ripening process, since many fibers like cellulose, hemicellulose, lignin, but especially pectin, are used as source of fibers hydrolyzed in the ripening process of the fruit [178].

Ash plays an important role in food analysis since the amount and type of minerals are established by this parameter and also, plays an important role in the delay the growth of microorganisms [179]. Our values were slightly higher than the previous studies, which suggests that avocado could be used as an alternative source of these minerals.

A big portion of human diet is related to macronutrients where carbohydrates are included, have an important role in many metabolic processes, especially in nourishment [180]. The variation in our results could be associated to environmental, climate and soil conditions, which affect the metabolic processes in the plant, resulting in a deficient performance of these processes [179]. The sugars and titratable acidity are relatable since they share almost an inverse proportionality in the ripening process, with an increase in the sugar (energy) content of fruit and a decrease in the TA (organic acids), which are required

in many metabolic processes [181]. The decreased shown in our most mature cycle (third harvest cycle) cloud be a consequence of the ripening process, since in addition to maturing on the bench, it was also placed in the refrigerator, which causes a slowdown in the ripening itself and in the different metabolic processes, resulting in a decrease in sugars and an increase in organic acids [181].

In fruits and vegetables, the green color is one of the most desirable characteristics caused by chlorophylls which are susceptible to degradation from heat or acidic conditions [182]. Many studies analyzed the effect of pH conditions in chlorophylls content, leading to the conclusion that alkaline conditions are more suitable for preventing degradation of the green color [183]. This finding is relatable to our observations, as the acidic nature (pH<7) of our varieties does not enhance the concentration of chlorophyll a and b, which intend to be higher at higher pH levels. The same happen with other pigments very important in food industry, the carotenoids.

In Fig. 19 a heatmap of the proximate analysis demonstrate the distribution of the parameters across cycles and in the different tissues. Its notable that one or two varieties stands out in each parameter, such as the CB variety in protein, the RCG and CB in fiber, the RCG in sugars, and the RCF in lipids. It is interesting that the commercial variety does not appears in higher content in any parameters, which is indicative that our regional varieties could and should be used more than commercial ones.

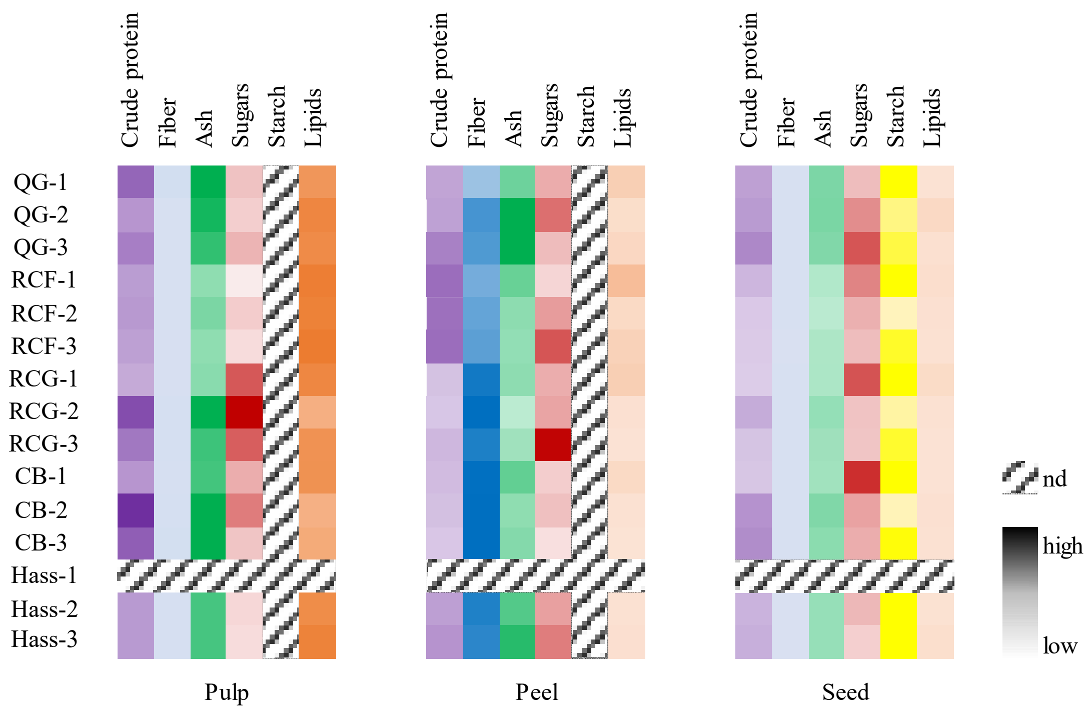


Fig. 19 Proximate analysis of the avocado samples. Color gradient (low to high) represents the relative concentration levels expressed in g/100g. Figure from author.

3.6. CIELab Color

The property color can be utilized as a quality indicator measure of the fruit while still on the tree, but most importantly in the evaluation of the ripening process [184]. The analysis consisted in the evaluation of the three coordinates color values, *L**, *a**, and *b**.

Our *L** values, that measures the lightness of the color, ranged between 52.55 to 82.37 in the pulp, while in the by-products, it oscillated between 18.38 to 57.10 in the peel and 53.23 to 69.38 in the seed. It was demonstrated that the peel was the darkest tissue, meanwhile the pulp achieved the lighter color values. Notably, the RCF variety was the one that displayed the lower *L** values in every tissue, which was indicative that was our darkest variety.

The parameter *a**, which measures the redness (+*a**) and the greenness (-*a**), shown values ranging from -2.53 to -12.40 in the pulp, exhibiting a greenish color in all varieties. Curiously, the commercial variety *Hass* was the one with a less greenish color. In the by-products, the peel displayed values ranging between 5.35 to 12.81, while in the seed values oscillated between 9.90 to 15.21, being these by-products placed in a reddish color, more accentuated in the seed. It is important to highlight that, the QG variety was the one that scored the higher parameter values in all tissues.

In the *b** parameter, which measures the yellowness (+*b**) and blueness (-*b**), it was detected values spanning between 30.85 to 68.23 in the pulp, 21.10 to 52.87 in the peel and lastly 23.03 to 29.13 in the seed. All tissues displayed a yellowish color with more evidence in the pulp.

Ramtahal et al. [185], evaluated the pulp of *Pollock* avocado variety, where values of CIEL*a*b* parameters were 73.4, -1.7, and 43.7 for *L**, *a** and *b** respectively, which is comparable to our findings, except for *a** values, in which greenish was more evident in our results. In another study conducted in Colombian *Hass* avocados, were detected values of *L**, *a** and *b** of 38.58, -13.37, and 19.73, respectively, which was similar to our results only in the *a** parameter, while the other values stayed much lower than ours [184]. Other investigation performed by Aldoradin-Puza et al. [186] analyzed the fresh and thawed *Hass* avocado, achieved values of *L** ranging from 71.43 to 73.91, followed by -3.44 to -5.25 in *a** parameter, and 36.55 to 43.95 in *b** parameter, which were slightly lower than our findings, but still comparable. This was already expected since the drying process and the use of dry matter powered increased in these parameters, especially lightness [187].

In the Table 5, where small letters indicate significant statistical differences ($p \leq 0.05$) between the varieties throw tissues and harvest cycles. In the first harvest cycle, it

was observed in the parameter *L** of the pulp, a similarity between the QG and RCF varieties, while in the peel and seed, all varieties displayed differences in the first case, and similarity was achieved between the RCF and RCG varieties in the second case. In the parameter *a**, similarities were identified in the pulp between the QG and RCG varieties, as well as between the RCF and CB varieties. All the varieties exhibited distinctions in the peel within the by-products, while the RCF and RCG varieties reflected similarities in the seed. In the parameter *b**, the QG and CB varieties mirrored themselves with the RCG variety, showing similarities between them in the pulp, while the RCG and CB varieties showed similarities in the peel, and differences in all varieties were observed in the seed.

In the second harvest cycle, the parameter *L** in the pulp of the QG and RCF varieties demonstrated similarities, as well as in the CB and *Hass* varieties. In the peel, similarities were observed between the QG and *Hass* varieties, as well as between the RCG and CB varieties. In the seed, the QG and RCF varieties mirrored each other, as did the RCG and CB varieties.

In the parameter *a**, meanwhile all samples displayed differences in the pulp, the by-products exhibited the same pattern with differences in all varieties, except in the RCF and RCG that mirrored themselves. In the parameter *b** of the pulp, similarities were observed between RCF, RCG, and CB varieties, while in the seed, those similarities were achieved between the RCF and RCG varieties. In the peel, the QG variety shares similarities with the *Hass* variety, which, in turn, exhibits resemblances with both the RCG and CB varieties. The latter two also show similarities with the RCF variety.

In the last harvest cycle, in the parameter *L**, of pulp and peel showed differences between the varieties, with only two showing similarities, the RCF and RCG varieties in the first case, and the RCG and *Hass* varieties in the second case. In the seed, only differences across varieties were observed. In parameter *a**, all tissues displayed a consistent pattern with variation in all samples, except for the RCF and RCG, which exhibited similar patterns in the pulp. Additionally, QG and CB varieties showed similarities in the peel, and finally the RCG and *Hass* varieties displayed resemblances in the seed. In parameter the *b** of the seed, variations among all the varieties were detected. However, both pulp and peel exhibited similarities between the two varieties, specifically the RCF and CB in the pulp, and the QG and RCG in the peel.

In the Table 5, the capital letters identify the differences between cycles for each variety. The *L** parameter of the pulp, the QG, RCF, and CB varieties exhibited a consistent pattern, with the first harvest cycle differing from the subsequent ones. However, in the second harvest cycle of the RCG variety, distinct characteristics were displayed. The *Hass*

variety showed variations in both cycles. In the a^* parameter, the three mentioned varieties exhibited a uniform behavior, where the first harvest cycle differed from the subsequent ones. In contrast, the RCG and *Hass* varieties showed variations across all the harvest cycles. Regarding the parameter b^*, distinctions were observed among the QG and RCG varieties, throughout all the harvest cycles. However, in the case of the RCF and CB varieties, the first harvest cycle differed from the subsequent ones. No differences were detected between the harvest cycles in the *Hass* variety.

Concerning the peel, a significant variability was noted in the L^* parameter across the harvest cycles and the varieties. The QG and *Hass* varieties exhibited differences consistently throughout all the harvest cycles. In contrast, the remaining three varieties displayed distinctions in specific cycles. The RCF variety in the first harvest cycle, the RCG in the second harvest cycle, and the CB in the third harvest cycle. In the a^* parameter, differences were observed across all the harvest cycles for all the varieties. Conversely, in the b^* parameter, the *Hass* variety showed no distinctions between the harvest cycles. However, the QG and CB varieties exhibited differences in the first harvest cycle, while the RCF and RCG varieties displayed variations in the third harvest cycle.

The parameter L^* in the seed of the RCF and RCG varieties displayed differences in the third and first harvest cycles, respectively, while the other varieties showed differences between all the harvest cycles. In the parameter a^*, the *Hass* variety did not exhibited differences between the harvest cycles, conversely the QG, RCG, and CB displayed differences across all the harvest cycles. The first harvest cycle was different in the RCG variety. In parameter b^*, while the QG and *Hass* varieties lacked differences between the harvest cycles, the RCF and RCG varieties showed differences in the second harvest cycle, and in the CB variety the first cycle was the outsider.

In addition to the observed variability in parameters, harvest cycles, and varieties, it is noteworthy that most regional varieties exhibited higher values in the parameters with commercial interest. This suggests that, in this coordinate color system, the regional varieties possess a more pronounced and vibrant color, making them visually more appealing to the human eye and, consequently, to the consumer.

The first factor that consumers detect and evaluate in food industry is the color, and pH greatly effects it. Although alkaline conditions help prevent color degradation in fruits and vegetables, slighly acidic conditions (5 - 6.9) could maintain and stabilize the color parameters [183]. Our finding indicated that, despite some variability, higher acidic conditions may lead to increased variability, while more alkaline conditions could increase to much brighter and vivid colors in our analysis [183].

Table 5 Colorimetric evaluation in five different avocado varieties through different tissues.

Tissue	Parameters	Cycle	QG	RCF	RCG	CB	Hass
Pulp	L	1st	70.95 ± 0.38 ^{a,A}	52.55 ± 1.13 ^{b,A}	69.12 ± 1.05 ^{a,A}	77.09 ± 0.78 ^{c,A}	na
		2nd	68.87 ± 0.20 ^{a,B}	70.08 ± 0.40 ^{a,B}	75.91 ± 0.38 ^{b,B}	81.73 ± 0.42 ^{c,B}	81.54 ± 0.61 ^{c,A}
		3rd	67.80 ± 0.62 ^{a,B}	70.53 ± 1.09 ^{b,B}	70.40 ± 1.39 ^{b,A}	82.37 ± 0.54 ^{c,B}	79.39 ± 0.06 ^{d,B}
	a	1st	-11.66 ± 0.54 ^{a,A}	-9.50 ± 0.03 ^{b,A}	-10.70 ± 0.59 ^{a,A}	-8.91 ± 0.34 ^{b,A}	na
		2nd	-12.40 ± 0.18 ^{a,A}	-9.76 ± 0.70 ^{b,A}	-6.18 ± 0.58 ^{c,B}	-8.23 ± 0.17 ^{d,A}	-4.05 ± 0.57 ^{e,A}
		3rd	-6.26 ± 0.03 ^{a,B}	-4.85 ± 0.59 ^{b,B}	-4.47 ± 0.12 ^{b,C}	-3.70 ± 0.56 ^{c,B}	-2.53 ± 0.02 ^{d,B}
	b	1st	43.82 ± 1.53 ^{a,A}	68.23 ± 6.07 ^{c,A}	57.08 ± 0.96 ^{ab,A}	48.54 ± 1.92 ^{a,A}	na
		2nd	55.15 ± 0.29 ^{a,B}	40.41 ± 2.06 ^{b,B}	37.92 ± 0.92 ^{b,B}	37.89 ± 0.82 ^{b,B}	31.24 ± 1.14 ^{c,A}
		3rd	50.86 ± 0.12 ^{a,C}	39.96 ± 2.69 ^{b,B}	46.24 ± 1.92 ^{c,C}	35.47 ± 1.79 ^{b,B}	30.85 ± 0.02 ^{d,A}
Peel	L	1st	34.32 ± 0.99 ^{a,A}	18.38 ± 0.30 ^{b,A}	50.21 ± 0.52 ^{c,A}	54.71 ± 0.58 ^{d,A}	na
		2nd	46.10 ± 1.84 ^{a,B}	29.46 ± 1.68 ^{b,B}	57.10 ± 0.30 ^{c,B}	55.30 ± 1.06 ^{c,A}	46.92 ± 0.61 ^{a,A}
		3rd	38.90 ± 0.09 ^{a,C}	30.06 ± 0.06 ^{b,B}	49.84 ± 1.09 ^{c,A}	47.04 ± 0.22 ^{d,B}	49.32 ± 0.68 ^{c,B}
	a	1st	8.64 ± 0.45 ^{a,A}	3.74 ± 0.31 ^{b,A}	8.47 ± 0.03 ^{a,A}	10.96 ± 0.10 ^{c,A}	na
		2nd	7.45 ± 0.44 ^{a,B}	5.35 ± 0.30 ^{b,B}	5.47 ± 0.21 ^{b,B}	8.98 ± 0.16 ^{c,B}	11.13 ± 0.10 ^{d,A}
		3rd	12.81 ± 0.07 ^{a,C}	6.40 ± 0.25 ^{b,C}	7.11 ± 0.25 ^{c,C}	12.72 ± 0.18 ^{a,C}	9.46 ± 0.23 ^{d,B}
	b	1st	52.87 ± 1.19 ^{a,A}	29.30 ± 0.66 ^{b,A}	32.69 ± 0.69 ^{c,A}	38.56 ± 0.44 ^{c,A}	na
		2nd	39.12 ± 2.96 ^{a,B}	27.43 ± 3.97 ^{b,A}	32.13 ± 0.38 ^{bc,A}	32.89 ± 1.13 ^{bc,B}	37.59 ± 0.25 ^{ac,A}
		3rd	41.09 ± 0.46 ^{a,B}	21.10 ± 0.80 ^{b,B}	21.84 ± 0.89 ^{b,B}	32.14 ± 0.43 ^{c,B}	37.38 ± 2.42 ^{d,A}
Seed	L	1st	56.53 ± 0.53 ^{a,A}	64.62 ± 1.33 ^{b,A}	63.53 ± 0.65 ^{b,A}	69.38 ± 0.32 ^{c,A}	na
		2nd	62.33 ± 0.48 ^{a,B}	62.32 ± 0.36 ^{a,A}	57.44 ± 1.22 ^{b,B}	58.55 ± 0.49 ^{b,B}	64.46 ± 0.40 ^{c,A}
		3rd	61.14 ± 0.23 ^{a,C}	53.23 ± 1.10 ^{b,B}	56.89 ± 0.05 ^{c,B}	63.04 ± 0.41 ^{d,C}	66.67 ± 0.20 ^{e,B}
	a	1st	15.21 ± 0.31 ^{a,A}	11.72 ± 0.34 ^{b,A}	11.97 ± 0.22 ^{b,A}	9.90 ± 0.25 ^{c,A}	na
		2nd	13.80 ± 0.12 ^{a,B}	15.14 ± 0.10 ^{b,B}	15.14 ± 0.42 ^{b,B}	12.03 ± 0.35 ^{c,B}	12.85 ± 0.16 ^{d,A}
		3rd	12.39 ± 0.03 ^{a,C}	15.66 ± 0.26 ^{b,B}	12.91 ± 0.08 ^{c,C}	10.67 ± 0.06 ^{d,C}	12.97 ± 0.08 ^{c,A}
	b	1st	26.72 ± 0.63 ^{a,A}	24.74 ± 0.64 ^{b,A}	23.03 ± 0.42 ^{c,A}	20.59 ± 0.30 ^{d,A}	na
		2nd	26.29 ± 0.13 ^{a,A}	29.13 ± 0.18 ^{b,B}	27.86 ± 0.94 ^{b,B}	23.12 ± 0.57 ^{c,B}	24.81 ± 0.23 ^{d,A}
		3rd	26.55 ± 0.06 ^{a,A}	25.53 ± 0.30 ^{b,A}	22.28 ± 0.11 ^{c,A}	23.97 ± 0.40 ^{d,B}	24.83 ± 0.19 ^{e,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 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 color parameter, in the distinct harvest cycles (years).; na- not analysed; nd- not detected.

3.7. Sensory Analysis

The sensory properties, such as appearance, texture, taste and aroma, can define the quality of the fruits and vegetables, and those are defined by every person based on its own preference and expectations, which in the case of avocado fruit, the degree of acceptation is closely linked to both flavor and texture [122].

In the Table 6, is described the sensory analysis grade of all the variety's flours, from avocado harvested in two stages, regular (second harvest cycle) and late (third harvest cycle).

Table 6 Sensory analysis evaluation. Grading from 0–5.

	Varieties				
	QG	RCF	RCG	CB	Hass
Regular harvest	2.6	2.2	1.8	2.5	3.8
Late harvest	4.3	3.0	3.0	3.5	3.8

In a first analysis, it was possible to observe that the varieties that were harvest late had the greater sensorial qualities, obtaining a higher acceptance by the panel of tasters. It is also interesting to note that in the case of the commercial variety *Hass*, there was no change in its score regardless of the harvest stage, while all the regional varieties increased their scores with the late harvest, and the QG variety even obtained the highest score in this parameter.

In this parameter, the sensory analysis was conducted for appearance, olfactory, flavor, and texture. In regular harvest, in the appearance characteristic, the QG and RCF varieties showed a lettuce-green color, while CB and *Hass* displayed a light-green coloration. The RCG variety obtained in its pulp a yellowish-green color. Related to olfactory characteristics, the QG and RCG varieties revealed a medium intensity and low persistence. Conversely, the RCF, CB, and *Hass* varieties showed low levels of both intensity and persistence.

In the QG variety, emerged an acidic and slightly buttery flavor, with high intensity and persistence. On the other hand, in the RCF variety featured metallic notes with high intensity and very high persistence. Acidic and peppery flavors very intense and persistence was obtained in RCG variety. Conversely, the CB variety showed slightly sweet and peppery flavors. The flavors in the *Hass* variety were characterized by sweet and buttery traits.

Related to texture, the flour of all varieties displayed a fine, soft, and dry to the touch, with buttery and creamy texture in contact to the mouth.

All varieties with the late harvest, all displayed the same characteristics related to appearance. The QG and RCF varieties revealed low intensity and medium persistence in olfactory trait, while the RCG, CB, and *Hass* obtained low levels of intensity and persistence.

Regarding the flavor, the QG variety allowed for the detection of *umami* notes, with a harmonious balance of acidity and sweetness, followed by a spicy aftertaste, which contributed to a well-rounded and complex profile for the flour. In the RCF variety, a quick change between sweet and acidic flavors was observed, accompanied by a persistent bitterness, which created a non-harmonious balance in this flour. Bitterness, peppery and acidic flavors were the notes that described the RCG variety, while with medium complexity and well-balanced blend of acidity and sweetness were the characteristics detected in CB variety. The *Hass* variety featured a slightly sweet flavor, with hints of citrus and pepper, conferring with a medium level of complexity in terms of taste. The same fine, soft, and dry flours to the touch, with buttery and creamy texture in the mouth, were obtained in this harvest stage.

After the evaluation of the sensory analysis parameters, an likability analysis was done using the hedonic scale (1-9 levels) raging from dislike extremely to like extremely, which finds the degree of liking or disliking of foods, across a large number of subjects (evaluation panel) [188].

Our findings showed a level 5 in the hedonic scale, corresponding to "neither like or dislike" which is normal when referring to dried products, since its characteristics (texture, color, odor, taste) change and could become less salient [189]. In a study performed with two varieties of Californian avocados (*Hass* and "3-29-5"), the *Hass* displayed a level 7 in the scale indicated a like moderately results, which is higher than our findings, but on the other hand the "3-29-5" variety obtained the same results as us [190]. On another research, with seven Mexican commercial and non-commercial avocado varieties, the levels obtained ranged between 5.6 to 7.2, which are slightly higher than our findings [191].

A general degree of purchase intention for avocado flour was obtained. The evaluation was determined on a scale of 1 to 5, going from "certainly wouldn't buy it" to "certainly would buy it", where avocado flours was placed in number 3, with the description of "Maybe I would buy it, maybe I wouldn't buy it". The same is to say that in this parameter the consumer's preference will dictate whether or not they purchase.

Sensory analysis is one parameter that is dependent or could be affected by many factors, including the carbohydrate concentration (sugar), which tend to increase with higher sugar concentrations [192]. The avocado low sugar content, and especially our varieties low content may have played a determining role in ensuring that our evaluation

was not bad, but also not good. Like other studies demonstrated, if we incorporate our flour products in the preparation of other products (cakes, breads, etc), we can obtain higher values in sensory analysis [193].

3.8. Total phenolic, antioxidant capacity and lipid content

3.8.1. Total phenolic content (TPC)

When talking about avocado fruit, pulp is known for its nutritional, biochemical and phytochemical characteristics that could impacts positively health, but also its by-products have a range of recognized bioactive properties, especially antioxidants that can promote human health and requires to be studied [144]. All tissues analysis regarding antioxidant is demand to observe the real antioxidant power of the fruit.

In total phenolic content (TPC), it is noteworthy that the by-products, namely the peel and seed, consistently demonstrated higher TPC values as (Table 7). In the first harvest cycle, the TPC of the pulp ranged from 0.93 to 1.55 mg GAE/g, whereas the peel exhibited a broader range of values, spanning from 11.36 to 20.90 mg GAE/g. Notably, the seed displayed the highest phenolic content, varying between 3.73 and 24.41 mg GAE/g.

In the second harvest cycle, there was a notable increase in TPC across all the tissue types. The peel recorded the highest TPC values, ranging from 22.38 to 46.86 mg GAE/g, followed by the seed with 6.47 to 41.23 mg GAE/g and, lastly, the pulp with a more limited range, spanning from 1.08 to 1.95 mg GAE/g.

Finally, in the third harvest cycle, a consistent uptick in TPC values was observed. Once again, the peel exhibited the most substantial TPC values, spanning from 10.98 to 48.47 mg GAE/g, followed by the seed with 4.19 to 33.44 mg GAE/g, and the pulp with 1.25 to 1.93 mg GAE/g.

Fan et al. [137], stated TPC in three rejected avocado pulp varieties, spanning from 0.15 to 0.21 mg GAE/g, lower than our finding. Similarly, Lyu et al. (2023) [78] recorded TPC values for three different avocado varieties' pulp, peel, and seed, ranging from 0.20 to 0.28 mg GAE/g, 29.22 to 77.85 mg GAE/g, and 26.93 to 44.91 mg GAE/g, respectively. These figures, in comparison to our findings, indicates lower TPC values for the pulp, but align closely with our results for the peel and seed.

Wang et al. (2010) [194] give TPC values from seven distinct avocado varieties revealing a range of 0.6 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. In contrast to our data, their values appear to be slightly higher for pulp and seed, but lower for the peel.

On the other hand, Runyogote et al. [195] reported TPC values ranging from 15.48 to 33.30 mg GAE/g in *Fuerte* avocado seed, very parallel to our findings. Numerous research studies have focused on assessing the phenolic content in various avocado tissues. Discrepancies in findings can be attributed to a range of factors, including ripeness, climatic conditions, storage parameters, geographic location, extraction solvents, and laboratory techniques. Given the favorable conditions for avocado cultivation on Madeira Island and the widespread consumption of regional avocado varieties, it is imperative to investigate their phenolic content.

3.8.2. Antioxidant capacity (DPPH)

Several antioxidant assays are employed to assess the antioxidant capacity of a wide range of food products [196]. Among these methods, the DPPH assay stands out for its sensitivity and lack for specialized equipment. Being a colorimetric technique, it relies on the reaction of DPPH, a stable free radical, with an antioxidant resulting in the production of a yellow hue [78,197].

In the first harvest cycle of our study, the antioxidant capacity of the pulp spanned from 0.35 to 0.88 mg AAE/g. In contrast, both peel and seed values exhibited a wider range, fluctuating between 78.43 to 90.41 mg AAE/g and 9.57 to 87.29 mg AAE/g, respectively. A general decline in the pulp values across most the varieties was observed in the second harvest cycle, with oscillations ranging from 0.40 to 0.67 mg AAE/g. On the contrary, the peel consistently showed an increase, ranging from 71.38 to 90.43 mg AAE/g. The seed values presented mixed results, with two varieties displaying an increase while in the others antioxidant activity decreases, resulting in values between 23.61 and 89.95 mg AAE/g.

Most of varieties in the third harvest cycle, show a notable decrease in both pulp and seed values, with ranges of 0.29-0.51 mg AAE/g and 14.22-88.85 mg AAE/g, respectively. Regional peel varieties exhibited mixed trends, but the *Hass* variety that ripened on the tree demonstrated an increase compared to the traditionally harvested *Hass*.

In comparison, Lyu et al. [78] disclosed antioxidant potentials of 0.08 to 0.16 mg AAE/g, 41.53 to 71.03 mg AAE/g, and 39.36 to 56.00 mg AAE/g for pulp, peel, and seed, respectively, which were typically lower values than our observations. Fan et al. [137] reported the pulp DPPH assay results between 0.12 to 0.32 mg AAE/g, which are lower than our findings. On the other hand, Wang et al. [194] documented values surpassing ours in all tissues, suggesting the solvent blend they used might be optimal for assessing the antioxidant capacity. As emphasized by numerous researchers, the antioxidant potential can be influenced by a myriad of factors, including the choice of the solvent.

3.9. Lipid content

In our investigation, we determined the lipid content using the methodology involving solvent extraction through the Soxhlet method, which is known for superior efficiency in lipid extraction when compared to alternative methods, such as supercritical fluid extraction [198].

The pulp showed the highest lipid concentrations, followed by the peel and seed. In the first harvest cycle, the lipid percentage ranged from 55% to 75% in the pulp, 7.5% to 27.6% in the peel, and 0.71% to 4.3% in the seed. A significant decrease in lipid content for pulp and peel was detected in the second harvest cycle, with readings of 36.8% to 70.5% and 1.8% to 7.8%, respectively. On the other hand, the seed lipid content increased to a range of 2.0% to 8.1%. In the third harvest cycle, the lipid level in pulp and peel rebounded, reaching 41.3% to 75.4% in the pulp and 0.6% to 12.7% in the peel, while the seed lipid percentages decreased to a range of 0.83% to 4.1%.

When it comes to this lipid parameter, interestingly, the RCF variety consistently produced the highest lipid content, in both pulp and peel throughout all the harvest cycles, surpassing even the commercial *Hass* variety. In the seed, on the other hand, the dominance oscillated between the RCF that lead in the first harvest cycle, followed by QG maturated in the tree, and *Hass* in second and third harvest cycles, respectively.

Diverging from prior investigations, Colombo & Papetti [144] stated seed lipid values of 4.6%, which slightly exceeded our results for the first and third harvest cycles, but fell short of our findings in the second harvest cycle. Their peel values, spanning from 4.4% to 9.1%, closely resembled our own. A study conducted by Salazar-López et al. [160], reported values of 2-9% for peel and 3-15% for seed, which mirrored our observations, although their seed results often surpassed ours. Vinha et al. [39], shows lipid values both in pulp (approximately 43.5%) and peel (around 2.2%), that generally lagged behind our findings, while seed values, at approximately 14.7%, exceeded our results. In relation to this research, it is essential to highlight that the lipid content in our avocados, especially in the pulp, exceeds the figures recorded in the avocado cultivated in Algarve. This emphasizes the remarkable diversity and richness of avocado varieties found on Madeira Island. Another study [165] presented values for pulp, peel, and seed of 64.09%, 2.18%, and 3.97%, respectively, which, for the most part, closely matched our findings.

Table 7 Total Phenolic Content (TPC), lipid content estimation, and antioxidant capacity (DPPH assay) in five varieties of avocado.

			Varieties				
Assay	Tissue	Cycle	QG	RFC	RCG M	CB	HASS
TPC (mg/g GAE)	PULP	1st	1.55 ± 0.01 ^{a}	1.09 ± 0.04 ^{a}	1.47 ± 0.03 ^{a}	0.93 ± 0.00 ^{a}	na
		2nd	1.08 ± 0.07 ^{a}	1.66 ± 0.06 ^{b}	1.74 ± 0.01 ^{b}	1.76 ± 0.08 ^{b}	1.95 ± 0.08 ^{b}
		3rd	1.25 ± 0.02 ^{a}	1.85 ± 0.09 ^{b}	1.88 ± 0.09 ^{b}	1.93 ± 0.01 ^{b}	1.75 ± 0.01 ^{b}
	PEEL	1st	20.90 ± 0.56 ^{a}	17.94 ± 1.13 ^{ab}	11.36 ± 0.14 ^{bc}	13.51 ± 0.88 ^{c}	na
		2nd	33.32 ± 0.21 ^{ab}	30.84 ± 2.43 ^{ac}	22.38 ± 1.04 ^{c}	46.86 ± 2.60 ^{b}	25.08 ± 2.13 ^{ac}
		3rd	10.98 ± 0.36 ^{a}	48.47 ± 2.72 ^{b}	32.39 ± 0.75 ^{bc}	11.96 ± 0.74 ^{a}	35.59 ± 1.84 ^{c}
	SEED	1st	3.73 ± 0.50 ^{a}	21.66 ± 3.74 ^{b}	21.78 ± 0.47 ^{b}	24.41 ± 3.02 ^{b}	na
		2nd	41.23 ± 0.62 ^{a}	13.84 ± 0.84 ^{b}	6.78 ± 0.12 ^{c}	6.47 ± 0.25 ^{c}	19.40 ± 0.13 ^{b}
		3rd	33.44 ± 2.84 ^{a}	4.19 ± 0.16 ^{b}	5.61 ± 0.78 ^{b}	15.24 ± 1.05 ^{c}	12.82 ± 0.85 ^{c}
DPPH (mg/g AAE)	PULP	1st	0.52 ± 0.04 ^{a}	0.35 ± 0.01 ^{b}	0.88 ± 0.02 ^{c}	0.86 ± 0.00 ^{c}	na
		2nd	0.43 ± 0.02 ^{ab}	0.54 ± 0.01 ^{b}	0.40 ± 0.04 ^{a}	0.67 ± 0.00 ^{c}	0.42 ± 0.02 ^{ab}
		3rd	0.44 ± 0.00 ^{a}	0.48 ± 0.03 ^{b}	0.29 ± 0.01 ^{c}	0.51 ± 0.03 ^{b}	0.46 ± 0.01 ^{a}
	PEEL	1st	85.65 ± 0.39 ^{a}	78.53 ± 4.74 ^{a}	90.41 ± 0.20 ^{a}	78.43 ± 6.39 ^{a}	na
		2nd	90.43 ± 0.28 ^{a}	89.42 ± 0.91 ^{a}	71.38 ± 1.13 ^{a}	87.04 ± 0.36 ^{a}	89.35 ± 0.32 ^{a}
		3rd	61.84 ± 2.13 ^{a}	89.47 ± 0.37 ^{b}	90.14 ± 0.61 ^{b}	68.54 ± 1.29 ^{ab}	89.62 ± 0.36 ^{b}
	SEED	1st	9.57 ± 1.13 ^{a}	87.29 ± 3.53 ^{b}	42.18 ± 2.09 ^{c}	84.24 ± 1.28 ^{b}	na
		2nd	89.45 ± 0.35 ^{a}	78.85 ± 7.74 ^{a}	53.82 ± 7.63 ^{a}	23.61 ± 0.43 ^{a}	88.82 ± 1.82 ^{a}
		3rd	88.85 ± 0.46 ^{a}	23.44 ± 0.41 ^{bc}	14.22 ± 1.12 ^{b}	53.20 ± 4.81 ^{cd}	40.41 ± 1.97 ^{bcd}
Lipids (mg/g)	PULP	1st	557.78 ± 0.59 ^{a}	730.99 ± 1.39 ^{a}	633.16 ± 0.57 ^{a}	593.28 ± 1.17 ^{a}	na
		2nd	669.52 ± 0.66 ^{a}	708.87 ± 0.56 ^{a}	379.03 ± 0.94 ^{b}	368.17 ± 2.46 ^{b}	628.80 ± 4.75 ^{a}
		3rd	635.85 ± 0.08 ^{ad}	754.20 ± 1.02 ^{b}	587.27 ± 2.19 ^{a}	413.42 ± 1.04 ^{c}	698.38 ± 0.98 ^{bd}
	PEEL	1st	153.70 ± 1.72 ^{a}	276.47 ± 2.29 ^{b}	155.13 ± 2.93 ^{c}	75.13 ± 1.42 ^{c}	na
		2nd	50.22 ± 0.00 ^{ab}	78.53 ± 2.81 ^{b}	24.23 ± 0.27 ^{a}	18.81 ± 0.38 ^{a}	21.42 ± 0.27 ^{a}
		3rd	92.08 ± 1.23 ^{a}	127.60 ± 3.00 ^{a}	8.81 ± 0.15 ^{b}	6.17 ± 0.15 ^{b}	29.50 ± 0.27 ^{bc}
	SEED	1st	10.84 ± 0.27 ^{a}	43.21 ± 0.67 ^{b}	7.14 ± 0.15 ^{c}	8.82 ± 0.15 ^{a}	na
		2nd	81.94 ± 0.39 ^{a}	24.33 ± 0.23 ^{a}	20.33 ± 0.82 ^{a}	26.10 ± 0.29 ^{a}	14.09 ± 0.49 ^{a}
		3rd	27.67 ± 0.00 ^{ab}	20.00 ± 0.20 ^{ab}	8.27 ± 0.18 ^{b}	18.90 ± 0.16 ^{b}	41.24 ± 0.62 ^{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 ($^{a\text{-}d}$) 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.

3.10. PCA of the antioxidant analysis

The PCA of the antioxidant analysis (Fig. A1) showed a notorious separation between all the tissues. The first two principal components accumulated 80.00% of variance. Component 1 explained 52.39% of variance, with eigenvalues of 17.51, while component 2 explained 27.61% with eigenvalues of 9.23. The pulp was closely associated with the lipid parameter (as stated in the previous PCA analysis), meanwhile both the peel and seed were the tissues with higher antioxidant content (TFC and the DPPH). Between them, it was observed the higher antioxidant power of the peel, verified by its proximity to the vectors.

Regarding the PCA of the pulp, the first two principal components accumulated 99.39% of variance. Component 1 explained 97.57% of variance, with eigenvalues of 8.50, while component 2 only explained 1.82% with eigenvalues of 0.16. It revealed a good separation between the regional varieties and the commercial one, with the regional ones being clustered in the direction of the lipidic vector. We can also detect a "symbiosis" between the regional varieties, with the QG and RCF varieties being similar and the same goes for the RCG and CB varieties.

In the PCA of the peel, the first two principal components accumulated 96.19% of variance. Component 1 explained 80.92% of variance, with eigenvalues of 10.81, while component 2 explained 15.27% with eigenvalues of 2.04. The variability detected in both principal components, drove to a higher dispersion in the vectors of the parameters analyzed. The separation between the regionals and the commercial variety was observed, and the "symbiosis" stated in the pulp was present in the peel as well.

Even higher variability in the vectors were detected in the PCA of the seed. the first two principal components accumulated 90.94% of variance. Component 1 explained 75.13% of variance, with eigenvalues of 7.54, while component 2 explained 15.81% with eigenvalues of 1.59. We observed that the first harvest cycle was distinct from the others, and that the QG variety was separated from the other regionals varieties by showing higher lipid and DPPH content on the second and third harvest cycles. Like the other tissues, the commercial variety stayed far from the others, with lower seed antioxidant content.

3.11. Fatty acid profile

As detailed in the previously mentioned methodology, we obtained the fatty acid profiles of avocado oil through the utilization of petroleum ether extracts in conjunction with GC-MS analysis. By scrutinizing their mass spectra and cross-referencing them with

the NIST14 and WILEY 229 mass spectral libraries, we were able to detect and identify the individual fatty acids. This comprehensive GC-MS analysis facilitated the quantification of these fatty acids in terms of milligrams per gram of oil in the various samples. It is important to mention that, to the best of our knowledge, there have been no prior studies characterizing the fatty acid profiles of the *Persea americana* Mill. Species from Madeira Island, which conducting comparative analyses presents a unique challenge. Nonetheless, the extensive research on commercial varieties does provide some reference points and guidelines for evaluation.

3.11.1. Pulp

The main fatty acids encounter by GC-MS in the avocado pulp across all varieties and years is displayed in Table 8. In saturated fatty acids, palmitic and stearic acids were identified, while oleic acid emerged as the major monounsaturated fatty acid. The primary polyunsaturated fatty acids detected were linoleic and arachidonic acids. Throughout all cycles, the oleic acid consistently emerged as the predominant fatty acid, which aligns with the findings in the literature [199]. An exception was observed in the QG variety, where the linoleic acid slightly exceeded the oleic acid. Nevertheless, the oleic acid's presence, relative to the total fatty acids, spanned from 46.9% to 55.9% across the samples. Linoleic acid (16.7% to 20.5%) typically ranked as the second most abundant, followed by palmitic acid (3.8% to 11.4%), stearic acid (4.7% to 9.7%), and arachidonic acid (2.7% to 5.9%).

Galvão et al. [200] analyzed the fatty acid profiles of three avocado varieties (*Fortuna*, *Collinson*, *Barker*) across all the three tissues (pulp, peel, and seed). The average fatty acid percentages in the pulp closely resembled our results, with minor variations. Their oleic acid percentages spanned from 42% to 51%, which is comparable to our findings. However, they obtained higher values than us for palmitic acid, ranging from 20% to 36%, while their linoleic acid (12-19%) and stearic acid (0.5-2.3%) percentages closely mirrored our observations.

In another research study, the fatty acid profile of four avocado varieties, including three Malaysian varieties and the commercial *Hass* variety, was investigated. The local varieties were primarily composed of oleic acid (ranging from 43.65% to 51.22%), followed by palmitic acid (ranging from 26.41% to 30.37%), linoleic acid (ranging from 12.75% to 17.45%), and stearic acid (ranging from 0.27% to 1.56%). These findings generally agree with our results, except for the notably higher levels of palmitic acid observed in our local varieties. Conversely, the *Hass* variety was characterized by a rich content of oleic acid (62.3%), followed by linoleic acid (15.7%) and palmitic acid (14.8%) [201]. It is worth

highlighting that the fatty acid composition of our regional avocado varieties highly resembles the hierarchical distribution seen in the *Hass* variety, where oleic acid is the most abundant, followed by linoleic and palmitic acids. In Table 8, we can observe that in the first harvest cycle of the pulp, significant differences were obtained in oleic and linoleic acid. The pulp of the CB variety showed enrichment in both palmitic and oleic acid, whereas the QG variety exhibited significantly higher level of linoleic acid ($p \leq 0.05$). In the fatty acid composition of the second harvest cycle for the pulp substantial similarities were observed among the various avocado varieties, except for the oleic acid. While there were no discernible differences among the QG, RCF, and *Hass* varieties, notable distinctions emerge when comparing these three cultivars to the RCG. In fact, RCG accumulates 16 times more oleic acid than the commercial variety. On the other hand, the CB variety, with intermediate levels of oleic acid, does not differ significantly from the other varieties ($p > 0.05$). In the third harvest cycle for the pulp, significant variations were observed in the content of palmitic, oleic, and linoleic acids. Specifically, when it comes to palmitic acid, CB differs from almost all varieties, except the QG, which exhibits similarity to the other samples. QG and *Hass* contain similar amounts of oleic acid, which are the lowest among the studied samples, while the RCF presents the highest values for this compound ($p > 0.05$). Although the CB pulp accumulates less oleic acid than the RCF, it is more enriched in this unsaturated fatty acid when compared to the CB and *Hass* varieties. *Hass* avocados have the most enriched pulp in linoleic acid ($p \leq 0.05$). When comparing the regional varieties, the RCG and CB pulps are also enriched in this polyunsaturated fatty acid (PUFA) ($p \leq 0.05$), whereas the QG and RCF varieties have the lowest levels.

A statistical analysis (as shown in Table 3) ($p \leq 0.05$), utilizing the capital letters to represent columns for different fatty acids across various avocado varieties, reveals harvest cycle-dependent variations. Particularly, in the case of the QG variety, the levels of oleic and linoleic acids are significantly higher in the first harvest cycle, while palmitic acid increases from the second to the third harvest cycle. Nonetheless, stearic, and arachidonic acids exhibit no significant inter-cycle disparities. For the RCF variety, oleic, linoleic, and stearic acids remain relatively consistent across the harvest cycles, while palmitic acid varies between the second and third harvest cycles, decreasing and then increasing, respectively. In the second harvest cycle for the pulp of the RCG variety, an enrichment in oleic and arachidonic acids was observed. In the CB variety, palmitic and stearic acid levels display inter-cycle variations, while oleic acid content differs between the second and third harvest cycles. Linoleic acid levels remain stable across all three harvest cycles, however, the presence of arachidonic in only one cycle makes it difficult to draw a conclusive analysis.

Table 8 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 ($^{a-d}$) 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.

3.11.2. Peel

In the peel (Table 9), no changes related to oleic acid content was observed, with values spanning from 47.7% to 62.3% across the harvest cycles. The posterior alignment passes through the linoleic acid (15.6% to 30.3%), stearic acid (11.3% to 17.5%), palmitic acid (1.2% to 7.2%), and arachidonic acid (3.3% to 5.3%). A previous study that analyzed three avocado varieties across all tissues reported fatty acid percentages of 39.9% to 43.0% for oleic acid, 17% to 22.6% for linoleic acid, and 19.8% to 28.9% for palmitic acid [200]. Our findings align closely with these results, being the only exception the palmitic acid, which we obtained in lower quantities. In a separate study conducted by Takenaga et al. [202], the fatty acid composition of the peel and seed of commercial avocado varieties, namely *Hass*, *Fuerte*, and *Bacon* was examined. Their analysis revealed that the peel values ranged 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 a mere 0.1% for stearic acid. These results contrast with our findings, apart from palmitic acid, where their levels exceeded ours. This distinction is notable, because our research states higher values in regional varieties, while the aforenoted study centered on globally recognized and extensively studied commercial avocado varieties.

The data in Table 9 shows that, the first peel harvest cycle exhibited noteworthy disparities ($p \leq 0.05$) in linoleic acid levels among the various avocado varieties. In this regard, the QG distinctly stood apart from all the other varieties, which mirrored the observations made during the pulp harvest cycle analysis. While stearic acid also revealed significant differences between the QG and RCG, the remaining acids showed similar patterns. However, regarding the second harvest cycle, a different pattern became evident, with evident variations observed in all acids except linoleic acid. Notably, the *Hass* displayed the highest levels of palmitic acid among the avocado varieties. Furthermore, in terms of regional varieties, RCG exhibited heightened levels of palmitic acid when compared to RCF. Additionally, the CB variety demonstrated an enrichment of oleic acid, marking a notable divergence from the other avocado varieties, and a greater amount of stearic acid in comparison to the RCF and *Hass* varieties. Both the QG and RCG does not differ in stearic acid content from the other varieties, and arachidonic acid was undetectable in the *Hass* peel. The level of this PUFA is notably greater in the RCG peel when contrasted with the RCF variety. Nevertheless, when comparing these two varieties to QG and RCF, no significant differences were observed.

In third peel harvest cycle, significant variations were observed in the levels of palmitic, oleic, and stearic acids. In the second harvest cycle, the *Hass* variety stood apart

from the rest due to its elevated palmitic acid content. Although no differences were identified among the regional varieties. Notably, the CB peel exhibited an increase in oleic acid compared to all the other samples, particularly when compared with *Hass* variety. Furthermore, the CB peel contained significantly higher levels of stearic acid compared to the peel of the *Hass* and the RCF samples. The stearic acid levels in the QG and RCG did not differ from those in the other samples. All the varieties presented similar levels of arachidonic acid, except the RCF peel where it was not detected.

The statistical analysis ($p \leq 0.05$) of fatty acids of different avocado varieties and harvest cycles exhibit distinct patterns. Palmitic acid remains consistent across all varieties and harvest cycles, albeit with minor values fluctuations. Oleic acid exhibits variations primarily based on harvest cycles. The QG shows differences between the first and second cycles, while the RCF, RCG, and CB each display unique patterns in the second harvest cycle. Linoleic acid's variation is exclusive to QG. Stearic acid demonstrates variations in the QG, RCF, and CB, but maintains consistency between the first and second harvest cycles. Arachidonic acid variates across all varieties, possibly due to fluctuations in quantities. In general, the second harvest cycle of the peel distinctly deviates from the other harvest cycles in terms of these differences.

Table 9 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≤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.

3.11.3. Seed

The seed composition (Table 10), once more the oleic acid remains the most predominant fatty acid, with percentages ranging from 54.5% to 69.4%. It is followed by linoleic acid (14.3% to 17.3%), stearic (7.4% to 13.1%), palmitic acid (1.8% to 11.8%), and finally arachidonic acid (1.6% to 3%).Takenaga et al. [202] investigated the lipid and fatty acid composition in the pulp and seed of three avocado varieties, and their findings differ from ours, both in terms of actual percentage values and the ranking of fatty acid quantities. In their research, linoleic acid was the most abundant, with values ranging from 35.3% to 38.2%, followed by oleic acid (22.4% to 24.1%), palmitic acid (17.7% to 19.0%), and stearic acid (0.3% to 0.8%). Another study conducted by Galvão et al. [200] analyzed the fatty acid profiles of the pulp, peel, and seed of three avocado varieties across. Their results exhibit minor differences with our findings, specifically in the seed, where the linoleic acid was the predominant fatty acid, with values spanning from 23.95% to 29.38%, followed by the palmitic acid (12.64% to 22.41%), the oleic acid (10.88% to 17.59%), and the stearic acid ranging between 0.94% to 2%. Both researches noted higher levels of *omega*-6 fatty acids in the seeds, but lower levels of *omega*-9 fatty acids when compared to our results.

Examining the seeds (lowercase letters along the rows) in a closely way to the pulp and the peel, some significant differences ($p \leq 0.05$) were registered between the varieties in the first harvest cycle. Notably, the RCG variety showed an enrichment in linoleic and stearic acid when compared to all the other varieties.

Regarding to the second harvest cycle, disparities were only detected in the oleic acid levels. The seeds of the QG variety contained more oleic acid than all the other varieties, except for the *Hass*. On the other hand, the CB seeds had the lowest oleic acid content. The oleic acid levels in the RCF and RCG varieties did not significantly differ from either the CB or *Hass* seeds.

In the third harvest cycle, fluctuations among varieties were evident solely in the oleic acid content. In this case, both the QG and CB varieties exhibited equal and significantly higher oleic acid levels compared to the other two varieties, namely, the RCF and RCG, which showed identical oleic acid content.

In the Table 10 capital letters, indicate significant variability in values for the QG variety. Notably, there is a distinct difference in oleic acid values between the first harvest cycle and the subsequent two harvest cycles. Moreover, in the case of linoleic acid, the increase in this compound sets the third harvest cycle apart from the others. The first and third harvest cycles differ from each other in the stearic acid levels, while the second harvest

cycle aligns more closely with the others. Among the other varieties, variations are detected in just one fatty acid for each. Specifically, in the RCF variety, the second harvest cycle differs from the others in terms of oleic acid. In the case of the RCG, the first and third harvest cycles differ from each other, while the second harvest cycle resembles them in linoleic acid levels. Regarding the CB variety, variations are observed in stearic acid, with the first harvest cycle deviating from the subsequent cycles.

Table 10 Avocado fatty acid composition (mg/g) performed by GC-MS of the seed of all five varieties.

Cycle	Fatty acid	Varieties 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-d) along the row indicates significant statistical differences between varieties (Tukey HSD, p≤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

3.12. PCA of the fatty acids

In the PCA of the fatty acids (Fig. A1) in all tissues, a separation between the regionals and the commercial variety was observed. The first and third harvest cycles showed more resemblance with the vectors of the fatty acids going in the same direction, while the second harvest cycle went through other quadrant.

In the PCA of the pulp, the first two principal components accumulated 91.08% of variance. Component 1 explained 62.89% of variance, with eigenvalues of 3.73, while component 2 explained 28.19% with eigenvalues of 1.67. The already expected separation between the regionals and commercial varieties were verified. Following the previous PCA, the first and third harvest cycle had a strong correlation with the same vector direction, while the second harvest cycle went in a different quadrant. It was observed two groups between the regional varieties, one composed by the QG and RCF varieties and the second by RCG and CB varieties, being this last, closely associated to fatty acids from the second harvest cycle.

In the PCA of the peel, the first two principal components accumulated 92.98% of variance. Component 1 explained 80.50% of variance, with eigenvalues of 4.47, while component 2 explained 12.48% with eigenvalues of 0.69. A more pronounce variability in the vectors of the parameters were detected, following the same distribution as the pulp. Among regional varieties, the variety CB stands out due to its separation from the other regionals ones, by showing higher fatty acids content.

For the PCA of the seed, the first two principal components accumulated 95.36% of variance. Component 1 explained 78.98% of variance, with eigenvalues of 7.29, while component 2 explained 16.38% with eigenvalues of 1.51. It stands out from the others by its enormous variability, not only in the vector of the parameters analyzed, but also in the distribution of the varieties in the graphic. Apart from the chronic separation of the commercial variety in relation to the regional ones, there was a record of a variability in the regional varieties, one being associated with a specific cycle. The QG variety was more associated to fatty acids from the third harvest cycle, while the RCF and RCG varieties were more related to the second and first harvest cycle, respectively.

3.13. Interactions between unsaturated fatty acids

The stated variability in fatty acid profiles (Fig. 20), ranging the pulp, peel, and seed across different harvest cycles, could be assigned to geographical variations and climate

conditions, since the RCF, RCG, and CB varieties came from the eastern part of the island, while the QG and *Hass* varieties were originated from the southern region.

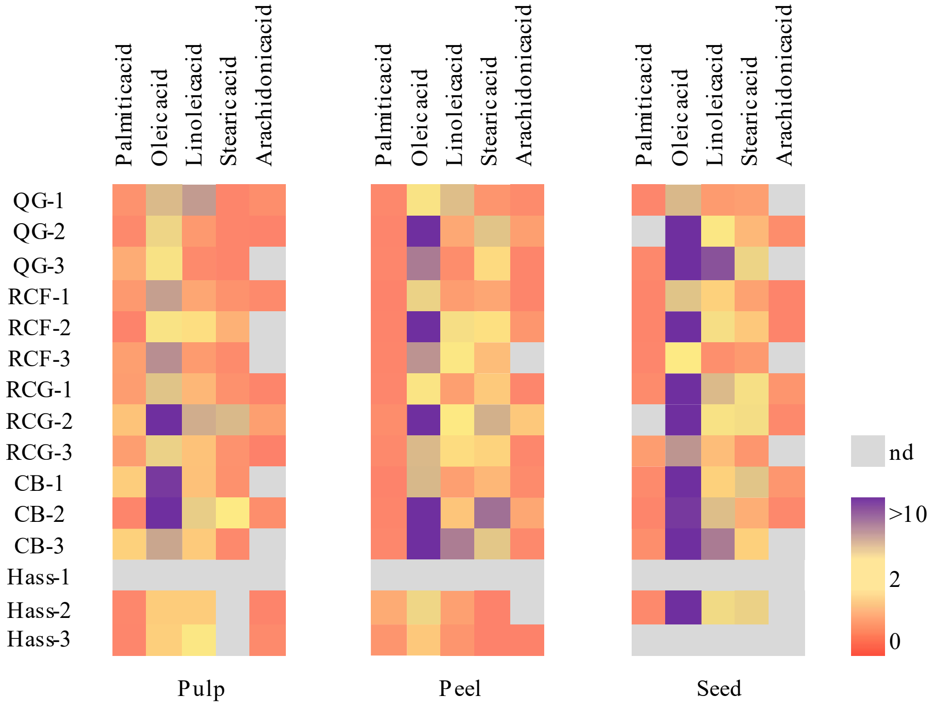


Fig. 20 Fatty acids concentration of avocado samples. Color intensity shows concentration levels (mg/g). Figure from author.

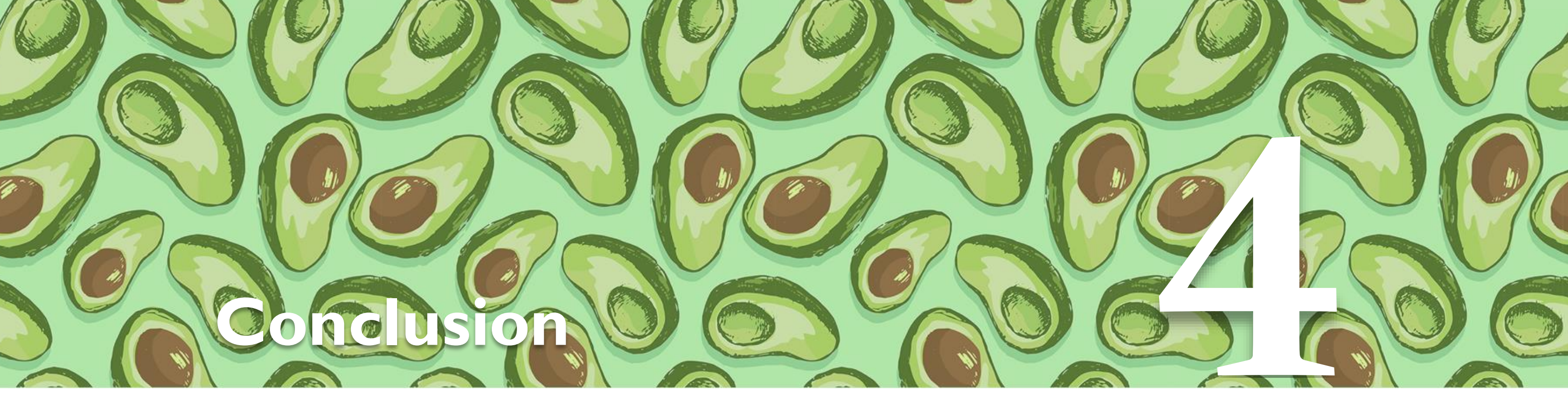
Various regions experience distinct annual weather patterns, and even within the same region, yearly climate variations can occur. These oscillations have a direct impact on the soil conditions, as indicated by Ferreyra et al. [203]. This theory aligns with the findings [203–205], who all concluded that geographical and climatic conditions influence fatty acid composition, highlighting the particularity that colder climates tend to yield higher quantities of oleic acid, which is the primary fatty acid in avocado fruit. Our results are consistent with this pattern, as the eastern varieties, grown in a colder climate compared to the southern ones, exhibit significantly higher oleic acid content in the pulp.

One gold finding in our research is the detection of arachidonic acid. This fatty acid is essential in our diet because animals, including humans, are unable to synthesize it. In the realm of agriculture, arachidonic acid in plants triggers defense responses against phytopathogens, leading to the accumulation of secondary metabolites such as polyphenols [109,111]. It's important to note that the literature suggests that arachidonic acid is rarely found in higher plants, as is the case here [111]. This discovery highlights the unique quality of regional avocados.

Polyunsaturated fatty acids (PUFAs) hold significant importance within the realm of fatty acids due to their vital role in human growth and overall health. They play a decisive role in determining indices that assess the effects of fatty acids on cardiovascular disease,

specifically the atherogenicity and thrombogenicity indexes, which provide insights into a plant's nutritional value [206]. The atherogenicity index (AI) determine the ratio between specific saturated fatty acids (namely C12:0, C14:0, and C16:0) and unsaturated fatty acids. This index offers insights into the tendency of lipids to adhere to cells within the circulatory and immune systems. Conversely, the thrombogenicity index (TI) calculates the relationship between the aforenoted pro-thrombogenic saturated fatty acids and anti-thrombogenic fatty acids, including monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), encompassing both *omega*-3 and *omega*-6. The TI provides an indication of the predisposition to form blood clots within blood vessels [141].

Lower values for both AI and TI indices indicate better cardiovascular health. Our study reflects these findings, showing values comparable to those found in traditional nuts, possessing elevated levels of unsaturated fatty acids, our avocados positively impact both atherogenicity and thrombogenicity indices. The low indices in nuts oils are linked to health benefits, like our varieties of avocados that mirrored this low levels. Specifically, traditional nuts typically exhibit low AI indices ranging from 0.07 to 0.14 and TI values between 0.16 and 0.35, as highlighted by [207].



4 Conclusion

The main objective of this study was to analyze and establish a nutritional, biochemical, and phytochemical profile of regional avocados varieties and compare them to a commercial one, or in other words, was to add-value the avocado fruit and its by-products to comprehend the differences across the regional varieties in terms of tissues and harvest cycles and compare them to the commercial variety.

Our analysis covered several parameters, in which some variability was detected but was clear that our results slightly surpassed those typically reported in the literature, potentially due to Madeira Island Mediterranean climate. In nutritional, pigments, and physico-chemical analysis, it was evident the association of tissues to specific parameters, with pulp, peel and seed, closely associated to lipids, crude fiber and starch respectively. Also in lipid content, probably the most important parameter in avocado, the RCF variety stands out surpassing the other regionals and the commercial varieties. Our finding revealed differences in these parameters across harvest cycles, with some differences between regional and commercial varieties, in which, the regional varieties displayed the best values for these parameters when compared with the commercial variety, being an indicator of the quality of regionals varieties. Additionally, it works as an enhancer for improvement and utilization of these cultivars compared to commercial varieties.

The same variability and differences between tissues and cycles happened with the antioxidant and the fatty acids analysis. The by-products (peel and seed) in this study revealed their remarkable antioxidant content, with the peel ranked as the tissue with highest antioxidant capacity. The major fatty acid founded in all samples was the monounsaturated fatty acid, oleic acid, with the remarkable presence of the arachidonic acid in the regional varieties, which is an uncommon occurrence in higher plants. In all parameters of on-tree maturation (the third harvest cycle) seemed to increase the concentration of certain parameters, being registered almost ever in regionals varieties.

In the sensory analysis the attribution of level 5 "neither like nor dislike" ends up being appropriate since we are talking about a dry product, flour, and naturally does not

have the attributes of fresh fruit, which ultimately in this parameter the consumer preference will dictate the opinion about the product.

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. Nevertheless, while these promising results highlights on the potential of regional avocados from Madeira Island, its necessary 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.

4.1. Future work

As stated, it is imperative continuing the study of this varieties and the potential associated, such as to explore maturation on the tree to increase the bioactive characteristics of the fruit, with specific studies and more controlled harvest times. Study of nutraceutical properties of the oil from pulp, peel, and seed of avocado. Other techniques, such as HPLC, could complement this work in the evaluation of biochemical parameters, to detect the pattern of sugars, antioxidants, vitamins, and carotenoids.

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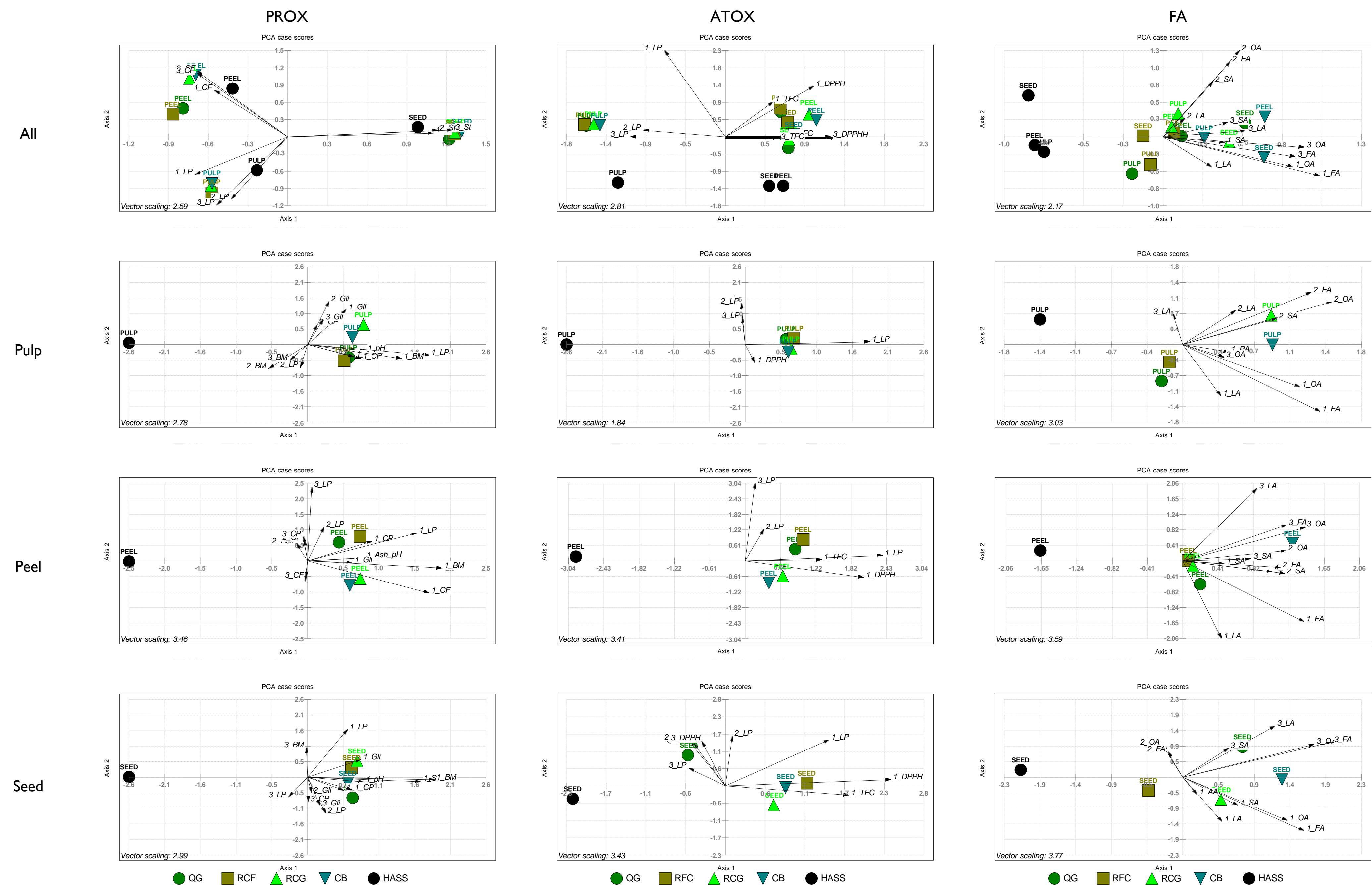
Table A1 PCAs of proximate analysis (PROX), antioxidant analysis (ATOX) and fatty acid profile (FA) of the different varieties of avocados across all tissues.



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