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Differences in Lipid Metabolism, Polar Metabolites, and Phenolics in *Persea americana* under Two Storage Conditions

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Abstract: Harvested avocado fruits can be potentially exposed both to pre- and post-harvest factors that may influence the susceptibility of fruit to quality loss. Regular air (RA) storage consists of approximately 78% nitrogen, 21% oxygen, 0.3% carbon dioxide, and smaller amounts of some other gasses. A controlled atmosphere (CA) is a changed atmosphere that is used to delay respiration and reduce fungal and physiological deterioration, and it is an appropriate and practical technology for prolonging the shelf life and maintaining the quality of fruits on a large scale. An experiment was conducted by collecting fruits from two different avocado orchards (Bartolillo and Quilhuica) during two harvest seasons based on dry matter content and two subsequent years. The current study was designed to investigate the changes in polar metabolites, phenolic compounds, and fatty acids in the skin of Hass avocados stored under two distinct conditions. Bartolillo orchard fruits were mostly correlated to linoleic and oleic acid as important variables. For Quilhuica, fruits were mostly correlated with palmitoleic, palmitic, and oleic acids. Phenolic content increased at the beginning of storage and decreased at the end of storage for one orchard and contrarily for others, indicating that the result was dependent on the orchard and storage condition. Serine, glutaric acid, xylitol and D-mannitol were the polar metabolites that most correlated with fruits of the Quilhuica orchard while ß-sitosterol and gluconic were related to fruits of the Bartolillo orchard. The differences in fatty acids, polar metabolites and phenolics were dependent on orchard and storage conditions.

Keywords: lipid metabolism; *Persea americana*; polar metabolites; phenolics; storage

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1. Introduction

Avocado is a highly nutritious and tasty fruit [1,2]. The market for avocado processing has increased significantly in recent years, and its value is expected to continue to increase beyond 2024 with new products entering the market, such as guacamole, frozen slices, sauces, purees, canned avocados, dried avocados, and avocado oil [2]. Increasing evidence of the health benefits of avocado is leading to increased consumption and stimulating research [3–6], not only on the potential health benefits but also on all other factors that may influence the external quality of the product in the final market. To extend the shelf life of fresh fruit after harvest, cold storage is the first issue, whether in combination with the controlled atmosphere or not. During storage, postharvest losses occur due to mechanical damage, diseases, and physiological disorders [7]. Important structural and metabolic components of plant/fruit cells include fatty acids and lipids. Changes in the lipid composition of the membrane frequently have detrimental effects on the cell's capacity to adapt to high temperatures and other stressful situations, which in fruit can result in a variety of physiological storage diseases [7].

Plant organ senescence, both naturally occurring and brought on by stress, has long been linked to loss of membrane function caused by lipid catabolism and peroxidation. The suggested senescence cascade of phospholipid catabolism involves two major enzymes: phospholipase D (PLD) and lipoxygenase (LOX), the former of which starts the process and the latter of which produces hydroperoxides from free di- and tri-en-oic fatty acids.

Both PLD and LOX are now known to comprise vast gene families that play a crucial role in responses to biotic and abiotic stress via the synthesis of lipid messengers and defense chemicals, aside from their damaging effects on membrane structure. Redistribution of phytosterols between the free and conjugated pools of sterols is another, less-researched modification in membrane structure related to stress responses in plants [8]. Plant polyphenols are a sizable class of specialized plant metabolites that can be further separated into several subclasses of important phyto-antioxidants, such as flavonoids and hydroxycinnamates. In other fruits, such as the tomato, it has already been noted that polyphenols tend to concentrate more in the fruit skin than in the pericarp because they play a part in how plants respond to stress [9]. In the post-genomic era, metabolite analysis, also known as metabolomics, is a crucial aspect of systems biology. All of a plant's main metabolites are polar substances. They include sugars, amino acids, organic acids, and a broad variety of other chemicals. In plant metabolism-related research initiatives, a quantitative study of such compounds is frequently necessary [10]. Understanding changes in fatty acids, polar metabolites, and phenolics during storage is key to improving and optimizing the current postharvest storage problems of Hass avocado. In this research, the main goals were to investigate changes in fatty acids, polar metabolites, and phenolics of Hass avocado fruits stored in two different storage conditions.

2. Materials and Methods

2.1. Sampling and Storage Conditions

Based on a prior pilot study that used ten orchards from various agroclimatic zones, two orchards were chosen [5]. Hass avocado fruit (400 per orchard) was collected during two harvests (2018/2019 and 2019/2020), which were characterized according to the dry matter as early 23–26% and late >27–30%. Fruit samples were collected and delivered to the laboratory facilities. The fruit was numbered before storage, and four small batches of 50 fruits each were randomly marked. Two hundred fruits were kept in regular air (RA) at 5 °C for 30 days (simulating the internal market storage) while the other 200 were kept in a controlled atmosphere (CA) at 5 °C, 4 kPa O_2, and 6 kPa CO_2 for 55 days (simulating export travel conditions to Asia). A total of 3200 fruits were evaluated. In RA storage, samples (50 fruits in each batch) were collected at harvest (day zero) and after 10, 20, and 30 days of storage, while in CA storage, samples were collected at harvest (day zero), 20, 35, and 55 days of storage. The fruit peel from each batch was removed, promptly freeze-dried under liquid nitrogen, and kept at −80 °C for later metabolic profiling study and other biochemical measurements.

2.2. Polar Metabolites

Polar metabolites (sugars, amino acids, and organic acids) were measured according to the Hatoum [11] methodology previously described by Uarrota [12]. Relative quantities were used to express the results. Briefly, the sample was incubated at 70 °C for 15 min with shaking after being mixed with 50 µL of cold methanol and 20 µL of 2910 ng L^{-1} phenyl-D-glucopyranoside, and then the supernatant was centrifuged (at 17,000× *g* for 20 min) and 100 µL was dried under nitrogen. Derivatization included methoximation and trimethylsilylation. Two GC-MS techniques were used, one for less concentrated chemicals, such as organic and amino acids, and the other for highly concentrated compounds, such as sugars. A volume of 1 µL of the sample was injected into the injector for each method, which had injector and interface temperatures of 220 °C and 280 °C, respectively. As a carrier gas, helium was employed at a constant flow rate of 1 mL min^{-1}. At a scanning rate of 2.66 scan cycles per second, mass spectra in the 50–600 m/z region were captured. Temperatures for the quadrupole and MS ion source were 150 °C and 230 °C, respectively. The procedure for more abundant chemicals included an injection with a split ratio of 1:150, and the oven temperature was set to begin at 120 °C (for 1 min), increase to 300 °C at a rate of 10 °C per minute, and then hold for 6 min. The splitless injection mode was utilized for the approach for less abundant compounds, and the oven temperature was set to begin at

50 °C (for 1 min), increase to 310 °C at a rate of 10 °C per minute, and then hold for 13 min. Using Mass Hunter Quantitative software, the chromatographic peaks were deconvolved and identified by comparing retention durations and mass spectra to a custom library of commercial standards and the NIST14 library (Agilent Technologies, Santa Clara, CA, USA). Using the peak area of phenyl β-D-glucopyranoside (as an internal standard), the sample fresh weight and a quality control (QC) sample representative of all samples, the peak area data were corrected to determine the relative response of each molecule discovered.

2.3. Fatty Acids

Following the earlier methodology described by O'Fallon [13] and Uarrota [14], fatty acid methyl ester production was carried out. In brief, freeze-dried peel samples were ground in a coffee grinder, and 1 g was then introduced to screw-cap culture tubes along with 5.3 mL of methanol and 0.7 mL of 10 M KOH in water. To properly penetrate, dissolve, and hydrolyze the sample, the tube was incubated in a 55 °C water bath for 1.5 h while being vigorously shaken every 20 min. A 0.58-mL volume of 24 M H_2SO_4 in water was added once the mixture had cooled to below room temperature in the cold tap water bath. By inversion, the tube was mixed before being incubated once again in a water bath at 55 °C for 1.5 h, shaking the tube every 20 min. The tube was chilled in a bath of ice-cold tap water following FAME synthesis. The tube was filled with 3 mL of hexane, vortexed for 5 min, centrifuged for 5 min at 3000 rpm, and the FAME-containing hexane layer was recovered and transferred to GC vials. Prior to GC analysis, the vial was sealed and stored at 20 °C. An automatic injection system-equipped Thermo Focus Gas Chromatograph (GC) was used to identify and measure the fatty acid mixture (AS3000auto-sampler). The following settings/parameters were applied: GC column: Varian CP-FFAP (free fatty acids), 25 m × 0.32 mm × 0.25 m, detector: FID at 280 °C, injection port temperature 250 °C, injection volume 1 μL, split ratio 1:20, column pressure 150 kPa helium. GC program: ramp 7 °C/min to 150 °C for 1 min, ramp 4 °C/min to 250 °C for 20 min, then hold at 250 °C. By comparing the peak regions of fatty acid standards, identification and quantification were performed. Injection errors were then fixed by using an internal standard (tetradecane) in each sample injected. Similarly, calibration curves for the various fatty acids found in avocados that have already been published were developed (oleic 18:1, palmitic 16:0, palmitoleic 16:1, stearic 18:0, linoleic 18:2, linolenic 18:3). Relative quantity was used to express the results.

2.4. Phenolic Contents

Kosinska's [15] colorimetric test with the Folin–Ciocalteu phenol reagent was used to measure the total phenolic content. In brief, a 100 mg ground avocado peel sample was extracted with 80% methanol in a thermostatic shaking water bath at 60 °C for 15 min. The supernatant was then centrifuged at 12,000× *g* for 10 min, filtered through 0.45 μm cellulose filters, and evaporated under N_2 flux. The dried extract was then dissolved in 1 mL pure methanol for further analysis. A 240-μL volume of distilled water, 20 μL of extract, 20 μL of 1 M Folin–Ciocalteau reagent, and 20 μL of 5%sodium carbonate made up the reaction mixture. The mixture was incubated for 30 min at room temperature, protected from light, and the absorbance at 765 nm was measured. Gallic acid (5–200 μg mL^{-1}, $y = 0.0012\ x + 0.002$, $r^2 = 0.99$) was employed as the standard, and phenolics were expressed as μg of gallic acid per gram of dry weight. Triplicate measurements were made.

2.5. Statistical Analysis

Data of polar metabolites was summarized and submitted to multivariate analysis to determine the significant variables related to storage condition and orchard. Data of fatty acids and phenolic contents were summarized and submitted to analysis of variance and expressed as mean and standard error of the mean. Principal component analysis was selected as the most effective method for dimension reduction, and all analyses were performed in R program version 4.2.2 [16].

3. Results and Discussion

3.1. Polar Metabolites

Analyses of polar metabolites are presented in the Figure 1A–D. Principal component analysis accounted 50.9% of total variance captured by the two first components, being 30.8% and 20.1% for component 1 (PC1) and component 2 (PC2), respectively (Figure 1C,D). Orchards were also better classified by polar metabolites and component 1. Serine, glutaric acid, xylitol, and D-mannitol were the metabolites that most correlated with fruits from the Quilhuica orchard. Contrarily, ß-sitosterol and gluconic were the compounds related to fruits from the Bartolillo orchard. Samples in the first component (Figure 1A) were most influenced by gluconic acid, tagatose, pentonic acid, xylose, hydroxybutanoic acid, glyceric acid, malic acid, xylitol, threonic acid, galactose, shikimic acid, citric acid, and chlorogenic acid. Samples in the second component (Figure 1B) were influenced by sucrose, glucitol, catechine, perseitol, epigallocatechin, glutamic acid, gluconicacid, xylitol, ß-sitosterol, serine, and mannitol.

Figure 1. (**A**–**D**): Variable contribution to PC1 and PC2 (**A**,**B**), the loadings (**C**), and principal component analysis biplot of polar metabolites (**D**).

The scientific field of metabolomics examines the intricate chemical signature of a biological system to determine its state. The term "metabolome" refers to any chemical species created, consumed, or present in the biological system that has a molecular weight between 1000 and 1500 Da. The direct results of gene expression, metabolism, dietary patterns, and environmental exposure are metabolites [17]. Because of the wide range of physicochemical properties that the metabolites have, comprehensive or untargeted metabolomics involves the entire compartment of the metabolites. Polar metabolite chemicals have also been observed to be impacted by variety, climate, and soil type [18]. In exploratory research using GC-MS, Hurtado-Fernandez [19] revealed, for instance, the

quantities of glyceric acid in ripening avocados. This study concluded that serine, glutaric acid, xylitol, D-mannitol, ß-sitosterol, and gluconic acid are highly changed during storage.

3.2. Fatty Acids

Results of fatty acids are represented in Figure 2A–L. Sample chromatograms are al so available as Supplementary Figure S1. Palmitic acid levels were observed to be higher at harvest and decreased during storage in both conditions and orchards evaluated (regular air–RA and controlled atmosphere –CA, Figure 2A,B), except in CA at day 35 for the Quilhuica orchard, for which higher levels were observed. Oleic acid (Figure 2C,D) increased in both storage conditions for the Bartolillo orchard, while for the Quilhuica orchard, oleic increased in the first days of storage, both in RA and CA, and then decreased. Iso-oleic acid (Figure 2E,F) increased for the Bartolillo orchard in both storage conditions, and for the Quilhuica orchard, an increase was observed until days 10 and 20 in RA and CA storage, respectively, followed by a decrease. A similar trend was observed foralpha-linolenic acid (Figure 2G,H). Linoleic acid (Figure 2I,J) decreased in RA storage for the Bartolillo orchard, while for the Quilhuica orchard, such a decrease was observed only until day 20, followed by an increase. In CA, a decrease was observed until days 20 and 35 for the Quilhuica and Bartolillo orchards, respectively, and then an increase until the last day of storage. Palmitoleic acid (Figure 2K,L) increased in RA for the Bartolillo orchard, while for the Quilhuica orchard, it increased until day 10 and then decreased. In CA, there was not a specific trend in palmitoleic acid. Analysis of variance of was performed for each orchard and independently of storage condition. Significant differences ($p < 0.05$, Tukey test) were observed for samples not stored and for those sampled during storage in RA for the Bartolillo and Quilhuica orchards. In CA, a significant decrease inpalmitic acid was found during storage except for the Quilhuica orchard, where higher levels were found at day 35 of sampling. Oleic acid significantly increased for the Bartolillo orchard in RA, while for the Quilhuica orchard, it increased until day 10 and then decreased. In CA, the differences were found only between days 20 and35 of sampling for the Bartolillo orchard, while for the Quilhica orchard, a typical trend was not found. Iso-oleic acid significantly increased for the Bartolillo orchard in RA, and in CA, it increased until day 20 and then decreased non-significantly. For the Quilhuica orchard, the trend was similar: an increase until days 10 and 20 for RA and CA, respectively ($p < 0.05$), and then a decrease. For alpha-linolenic acid, differences were according to storage days. Linoleic acid decreased significantly from day 10 in RA for the Bartolillo orchard, while in CA, a decrease was observed until day 35 of storage, followed by anincrease. Linoleic acid significantly decreased until day 10 in RA, and then significantly increased. A similar trend was observed in CA.

When the fatty acid data was submitted to principal component analysis (PCA), a better separation between the two orchards was observed. The total variance captured by the two first components (PCs) was 91.7%, being 84.8% and 6.7% for the first and second component, respectively (Figure 3A,B). The majority of Bartolillo orchard fruits were mostly correlated to linoleic and oleic acids as important variables. Most samples were grouped in the first component due to the presence of oleic, linolenic, iso-oleic, and palmitoleic acids, while those in the second component were most influenced by linoleic and alpha-linolenic acid (Figure 3C,D).

Avocados contain fruit oils rich in monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and saturated fatty acids, with 71% MUFA, 13% PUFA, and 16% saturated fatty acids (SFA). The level of monounsaturated oleic acid increases and the level of saturated fat decreases as the avocado fruit ripens [20].

Fruit of the Bartolillo orchard stored in RA condition presented increases in palmitoleic and oleic acids, while decreases in palmitic, linoleic, and linolenic acids were observed. Oleic acid levels increased during storage, and the authors noted that this fatty acid presents high-density lipoproteins and functions as an antioxidant [21].

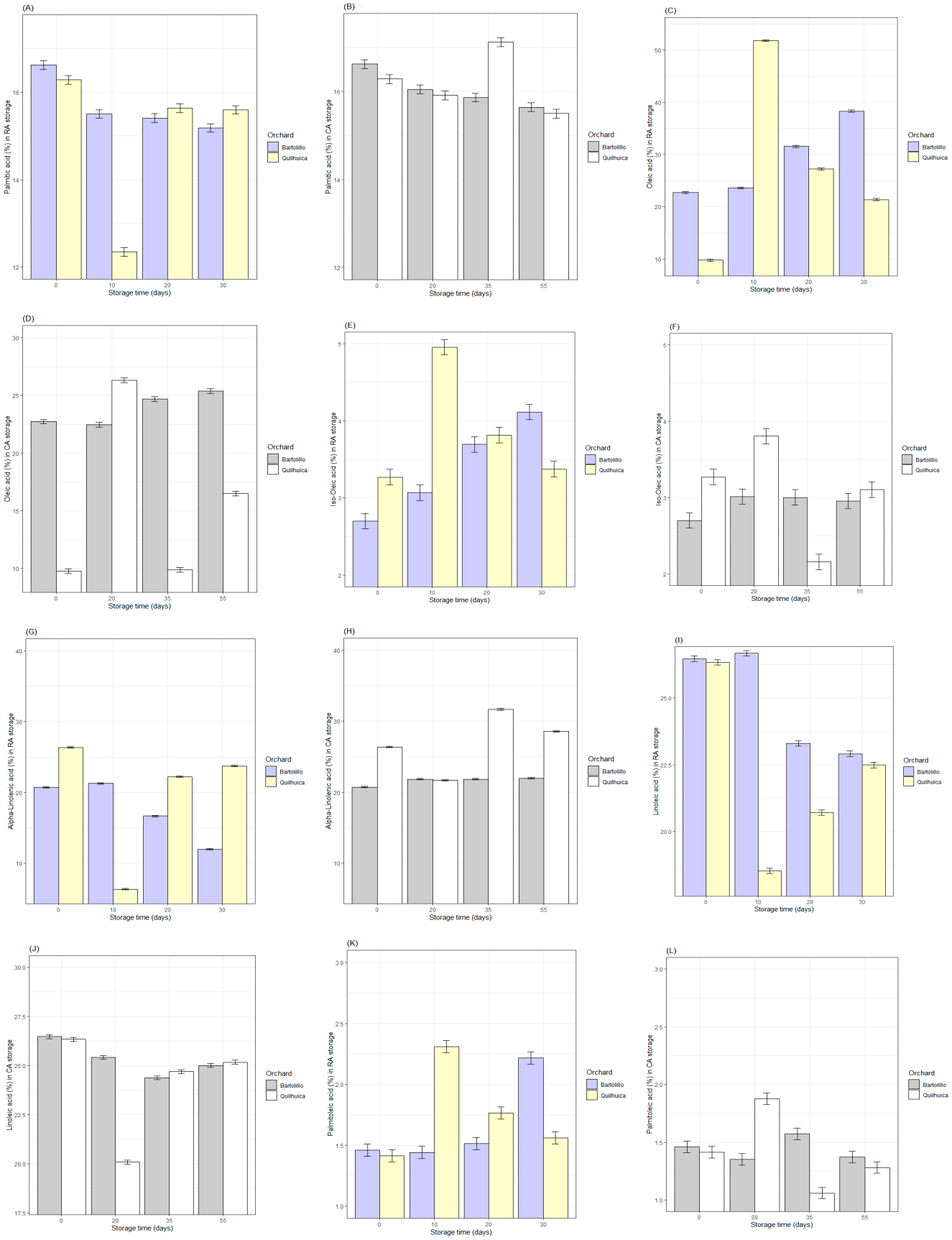

Figure 2. (**A**–**L**): Changes in fatty acids during two different storage conditions intwo Hass avocado orchards.

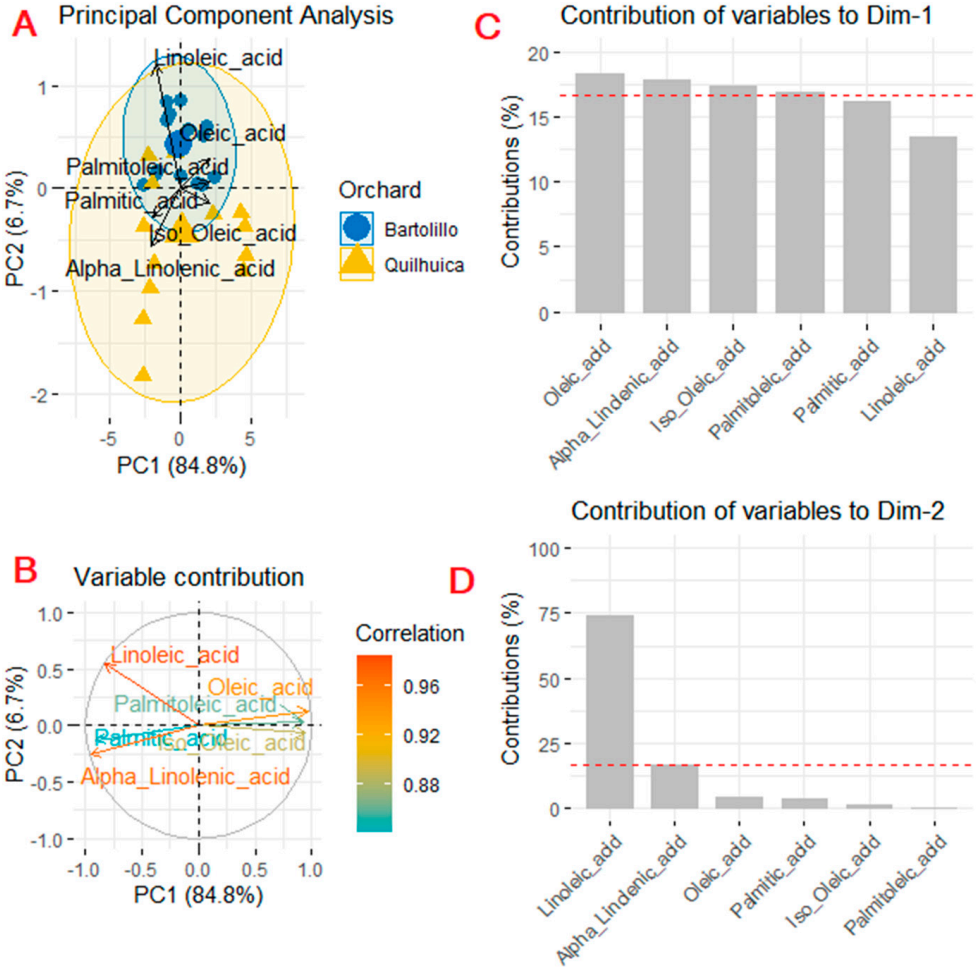

Figure 3. Principal component analysis (**A**), the loadings (**B**), and variable contributions to each component (**C**,**D**).

Linoleic (C18:2) and linolenic (C18:3) acid quantities were also shown to decrease during the storage periods [21]. When fruits were stored at 5 °C, Nahed [22] also observed a drop in linoleic and linolenic acids and an increase in the content of oleic acid. They claimed that the oxidation of unsaturated fatty acids into primary and secondary oxidation products was the principal cause of the decline in (C18:2) and (C18:3), while the decrease in linoleic and linolenic acids was mostly responsible for the rise in oleic acid. Only increases in palmitic and linolenic acid were seen in CA storage.

For the Quilhuica orchard, fruit stored in RA presented a higher increase in palmitoleic and oleic acids, while in CA, an increase was observed for palmitic and oleic acids. High-palmitoleic acid phenotype plants were shown to have a coordinated decrease in fatty acid synthase II activity and an increase in stearoyl-ACP desaturase activity, according to Salas [23]. According to certain theories, palmitoleic acid may stop beta-cell apoptosis brought on by saturated fats or glucose. It has been asserted that unsaturated fatty acids can withstand biotic challenges, such aspathogen infection and herbivore wounding, as well as abiotic factors, such as cold, heat, drought, and salt. They serve as components and regulators of cellular membranes in glycerolipids, a store of carbon and energy in triacylglycerol (TAG), stocks of components of the extracellular barrier, precursors of various bioactive molecules, and regulators of stress signaling, to name a few of their roles in stress defense [24].

3.3. Phenolic Contents

Figure 4A,B presents results of total phenolic contents during storage. As can be observed, in CA storage (Figure 4A), phenolics of the Quilhuica orchard significantly ($p < 0.05$, Tukey test) decreased until day 20, and then increased significantly until day 35, but non-significantly from 35 to 55 days of storage, while for the Bartolillo orchard, there was no typical trend. A significant increase was observed until day 20, and then a decrease until day 35. In general, there were significant differences during the storage.

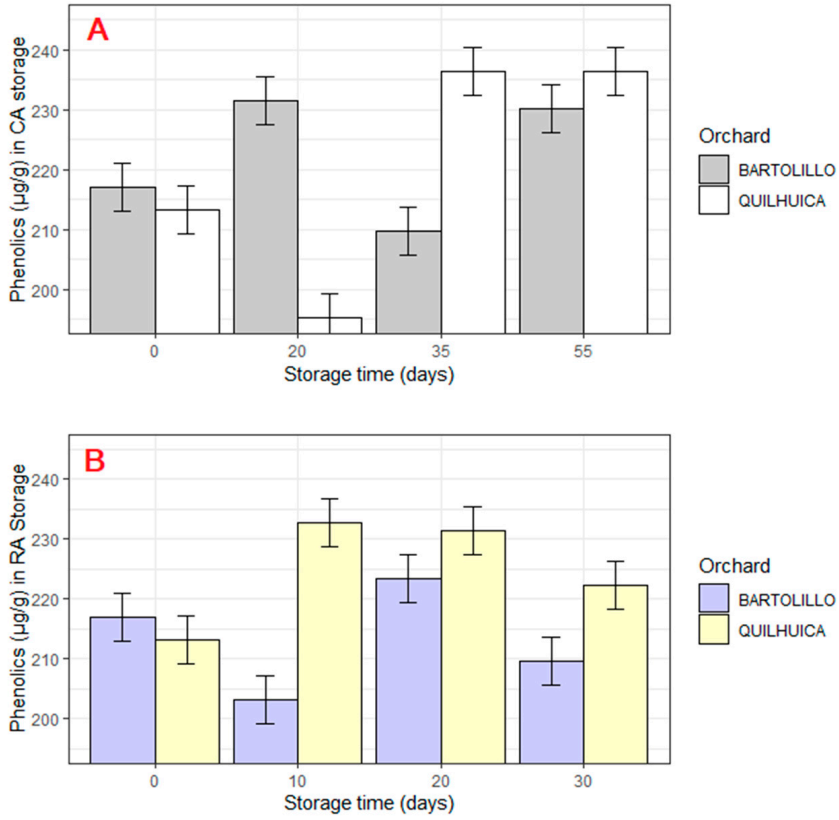

Figure 4. (**A**,**B**): Changes in total phenolic content in both orchards studied during both storage conditions (RA and CA).

In RA storage (Figure 4B), phenolics increased significantly for the Quilhuica orchard until day 20 and decreased on the last day of storage. There were non-significant differences between days 10 and 20 of storage, while for the Bartolillo orchard, there was again no trend observed. There was a decrease at day 10 and an increase at day 20, and finally a decrease on the last day of storage. The trend was according to the sampling day.

Fruits are a natural source of antioxidants since their phenolic chemicals are mostly responsible for their antioxidant activity. Numerous intrinsic factors, such as the genus, species, and cultivar, as well as extrinsic factors, including agronomic and environmental factors, handling, and storage, affect the phenolic content of food and plants [25].

Fruit's phenolic component composition may change depending on the climate and conditions after harvest, such as processing and storage. Processing and storage can cause phenolic compounds to undergo prolonged chemical and enzymatic oxidation, which helps reduce them [25]. On the contrary, Villa–Rodriguez [26] reported increases in phenolics.

According to the previous reports, the total phenolic content increased until the sixth day of storage before decreasing when senescence set in [25]. The decrease was related to a number of chemical and enzymatic modifications that take place throughout the fruit's rapid maturity phase. Glycoside hydrolysis by glycosidases, phenol oxidation by phenol oxidases, and polymerization of free phenols are some examples of these modifications [25]. The results of the present study are in accordance with those reported by Golucku and

Ozdemir [27], who reported that total phenolic content increased at the beginning of the harvesting period up to the second harvesting time and decreased at the end of the harvesting time (the third harvesting time). The phenolic composition of avocado fruits showed significant differences in terms of the cultivars and their storage conditions.

4. Conclusions

According to the orchard and storage conditions, fatty acids differed significantly. Linoleic and oleic acids were mainly associated with the Bartolillo orchard fruits. Palmitoleic, palmitic, and oleic acids were associated with the Quilhuica orchard. For one orchard, the phenolic content increased during first days of storage and decreased on the last days of storage, whereas the opposite was true for another, showing that the outcome was dependent on the orchard and storage conditions. The polar metabolites that were most closely associated with the fruits of the Quilhuica orchard were serine, glutaric acid, xylitol, and D-mannitol, whereas ß-sitosterol and gluconic acid were associated with the fruits of the Bartolillo orchard. The state of the orchard and storage of the fruits determines changes in lipid metabolism, polar metabolites, and phenolics during postharvest storage of Hass avocado.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020234/s1, Supplementary Figure S1 cited in the manuscript is available.

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Data Availability Statement: Datasets presented in this study are available to the user on request.

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Conflicts of Interest: The author declares no conflict of interest.

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