horticulturae

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Do Rootstocks Influence Global Fruit Quality, Postharvest Performance and Metabolite Profiles of *Persea americana* cv. Hass?

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Abstract: The choice of rootstock has a significant impact, not only on fruit growth and development, but also on avocado fruit quality and postharvest performance. The objective of this study was to evaluate and compare attributes related to the postharvest quality of Hass avocados from "Mexicola" and "Duke 7" rootstocks grown under similar conditions. This study included two harvests: early (23–26% dry matter) and middle (>26–30% dry matter) per season for the 2016/2017 and 2017/2018 seasons and two storage conditions (regular air (RA) at 5 °C and controlled atmosphere (CA) at 4 kPa O_2 and 6 kPa CO_2 at 5 °C) for 55 days. The results showed significant differences in firmness, color and vascular and flesh browning between storage conditions; in addition to these last three attributes, the rootstock played an important role. The fatty acid profile did not reveal significant differences between fruit from both rootstocks. Finally, the polar metabolite profiles revealed differences only for the storage condition, not associated to the rootstock, which could affect the postharvest performance of Hass avocado fruit. This study is one of the few available showing the interaction of rootstock/Hass cultivar on fruit quality and postharvest performance.

Keywords: fruit quality; heterogeneity; ripening; polar metabolites; *Persea americana* Mill.

Citation: Hernández, I.; Molina, V.; Fuentealba, C.; Alvaro, J.E.; Defilippi, B.G.; Pedreschi, R. Do Rootstocks Influence Global Fruit Quality, Postharvest Performance and Metabolite Profiles of *Persea americana* cv. Hass? *Horticulturae* **2023**, *9*, 184. https://doi.org/10.3390/horticulturae9020184

Academic Editor: Mohammad Zahirul Islam

Received: 6 January 2023
Revised: 25 January 2023
Accepted: 25 January 2023
Published: 1 February 2023

1. Introduction

Avocado production, demand and commercialization have significantly increased worldwide, in part due to it being considered as a superfood with health attributes. Avocado demand in international markets has expanded, and as a consequence for exporting countries (e.g., Chile, Peru, etc.), the challenges have increased regarding their ability to reach markets demanding up to 55 d travel, as in the case of Asia. The cultivar Hass dominates the international market, representing 95% of the fruit commercialized worldwide [1], and most of the fruit is commercialized in the formats "ready to eat" and "triggered" in the main consuming countries (e.g., Europe and the USA) [2,3]

As shipping days increase, avocado condition can be affected. The main quality problems reported for Hass avocado are related to fruit ripening heterogeneity in terms of color and firmness [4–7], increased incidence of rots and physiological disorders such as pulp and vascular browning [8], and during the last seasons, disorders such as black spots on the exocarp not associated with rots have also been reported [9,10].

Grafting is a well-known and well-used commercial practice in fruit production. Previous studies have shown that an appropriate combination of rootstock/scion is key in respect to nutrient uptake, water potential, plant vigor, fruit yield and even fruit quality [11,12].

There are numerous investigations evaluating the effect of a large number of rootstocks of different origins and characteristics on avocado crop yield (e.g., A8, A10, Barr Duke, Dusa™ (Merensky 2), Latas™ (Merensky 1), Duke 7, Reed, Rigato, SHSR-02, SHSR-04,

SHSR-05 Thomas, Toro Canyon, Velvick (Whiley), Velvick (Anderson), Velvick (Lynwood), V1, A10♀xVelvick, Velvick♀xA10, VC26, VC27, VC28, VC55, VC66, VC68, VC96, VC140, VC152, VC159, VC162, VC207, VC320, VC801, VC802, VC804, VC840, Degania 62, Nachlat 3, among many others) in which they have observed differences among rootstocks in their susceptibility to *Phytophtora cinnamomi* [13–15] and to *Verticillium* [16], in their mineral nutrient uptake [17,18] and in their response to salinity [19,20], among other characteristics.

Currently, the Chilean avocado industry relies on seedling rootstocks (mainly Mexicola and Zutano) and, to a much less extent, on vegetative propagated or clonal rootstocks (mainly Dusa® and Duke 7), which are being used in part due to their claimed greater potential in terms of productivity, uniformity and benefits such as their ability to adapt to stress factors, both pathological and edaphic [21]. Previous studies have reported the effects of rootstocks on avocado fruit quality, but they are limited to fruit and seed weight and shape, fruit oil concentration [22–24], anthracnose susceptibility [25] and internal quality such as flesh discoloration and pulp browning, potentially driven by their different capacities to absorb and transport nutrients to the fruits [26,27].

There is a need for the assessment of the effect of rootstocks on avocado cv. Hass on global fruit quality parameters (e.g., firmness, color, physiological and pathological disorders, etc.) and postharvest performance considering the extremely distant export markets and complete characterization at the metabolite level with a focus on key components (fatty acid profiles and polar metabolite analysis). Recently, Tietel et al. [12], by applying a complete metabolomics analysis, reported the impact of scion/rootstock reciprocal effects on fruit yield and biochemical fruit quality of *Citrus reticulata*. Thus, this research aimed (i) to evaluate the effect of two commercially used rootstocks for avocado cv. Hass in Chile on global fruit quality parameters and postharvest performance, simulating internal and distant markets, and (ii) to evaluate the effect of these two rootstocks on the key metabolite profiles (fatty acid and polar metabolite profiles) of avocado cv. Hass.

2. Materials and Methods

2.1. Experimental Design and Plant Material

This study was carried out at the Faculty of Food and Agronomical Sciences of the Pontificia Universidad Católica de Valparaíso (PUCV), located in the province of Quillota, Valparaíso Region, Chile, during the 2016/2017 and 2017/2018 seasons. Fruits were collected from the Experimental Station La Palma of PUCV. From a commercial plot, 20 cv. Hass avocado trees, of which half were grafted on "Mexicola" rootstock (seed propagated rootstock) and the other half were grafted on "Duke 7" (clonal rootstock), were randomly selected. Trees were grown under similar environmental and commercial crop management conditions. This area is characterized by a sandy clay loam soil and by a Mediterranean type of climate.

2.2. Postharvest Storage Experiments

The fruit, immediately after being harvested, were stored in a cold room at 5 °C. Two harvests corresponding to early harvest (23–26% dry matter) and middle harvest (>26–30% dry matter) for two seasons (2016/2017 and 2017/2018) were sampled. For each harvest, 400 fruits were collected from each rootstock, with an average weight of ~200 g (commercial weight). The fruit of each harvest (400 in total) were subjected to the following postharvest treatments: 200 fruits were stored in regular air (RA) at 5 °C for 30 and 55 days, respectively, and 200 fruits were stored in controlled atmosphere (CA) at 5 °C and 4 kPa O_2 and 6 kPa CO_2 for 30 and 55 days, respectively. Fruit (10 fruit per sampling point) were sampled during postharvest storage (harvest, 30 and 55 d) either in RA or CA for assessment of global fruit quality parameters such as mesocarp firmness, fruit color, ripening heterogeneity and physiological and pathological disorders. Fruit color index, physiological disorders and rots were only assessed after 30 and 55 d storage, because at harvest these problems were not present. After each storage period, 30 and

55 days, the remaining fruit were removed and subjected to 20 °C and 65% RH to determine ripening heterogeneity.

For the metabolite characterization (fatty acid and polar metabolite profile), only fruit from the early harvest of the 2016/2017 season were used. Fruit biopsies on each independent fruit were used as biological replicates and sampled, as described by Pedreschi et al. [4] at different storage days (0 and 30 d storage either in CA or RA), immediately frozen in liquid nitrogen and kept at −80 °C until analysis. The biopsies were sealed with petroleum jelly and wax, as previously described by Pedreschi et al. [4] and Fuentealba et al. [28].

2.3. Evaluation of Global Quality Parameters

2.3.1. Firmness

Fruit firmness was initially evaluated using a pressure gauge with a 4 mm plunger; as the fruit softened, an 8 mm plunger was used and, finally, at edible ripeness, a 12 mm plunger was used. The data obtained were normalized according to the type of plunger used. The two sides of the equatorial diameter of the fruit were measured to determine an average firmness, which was expressed in N.

2.3.2. Color and Physiological Disorders Severity

Color measurements were made based on a hedonic scale from 1 to 5: 1 = 100% green of the skin surface; 2 = 20% black/purple color over the green of the skin; 3 = 60% black/purple color over the green of the skin; 4 = purple on 100% of the skin surface; 5 = black on 100% of the skin.

The percentage of fruit with peel color change from green to black was calculated considering the percentage of fruit with a score between 3 and 5 at 30 and 55 days of storage. Both internal and external physiological disorders of the fruit were evaluated with a hedonic scale from 0 to 4: 0 = no occurrence; 1 = slight damage; 2 = moderate damage; 3 = moderately severe damage; 4 = severe damage [29].

2.3.3. Ripening Heterogeneity Assessment

Ripening heterogeneity was determined by analyzing 200 fruits of cv. Hass from each rootstock ("Mexicola" or "Duke 7") and storage conditions (RA or CA). One hundred out of the 200 fruit were removed at 30 d, and the remaining fruit were removed at 55 days of storage. Then, the fruit were placed at 20 °C with 65% relative humidity, and the number of days to reach edible ripeness (2–8 N) was recorded on each independent fruit. The color and severity of physiological and pathological disorders were also determined at edible ripeness, as described above. However, instead of the percentage of fruit with color change, the percentage of fruit that completely reached a black color on the skin (fruit with color values 4 and 5) was assessed.

2.4. Fatty Acid Profile Analysis

Fatty acid analysis was performed based on fresh mesocarp kept at −80 °C, using the biopsies collected of the early harvest of the 2016/2017 season, as previously described. The oil was extracted using the chloroform–methanol method of Bligh and Dyer [30]. Fatty acid methylation and quantification were performed following the methodology of Chirinos et al. [31]. The samples were analyzed in a gas chromatograph coupled to a flame ionization detector (GC-FID); helium was used as the carrier gas, and the detector was operated at 208 °C. The injection volume corresponded to 1 µL in split mode 1:50 at 250 °C. A HP-5MS (30 m × 0.25 mm × 0.25 um) column was used. The initial oven temperature was maintained at 50 °C for 1 min, and then increased to 170 °C (at a rate of 20 °C/min), and finally rose to 250 °C (to 3 °C/min) and maintained at this temperature for 1.3 min. Fatty acids were quantified and identified based on the calibration curves and retention times of external standards. Fatty acids were expressed as percentage fatty acid/g of oil sample. Because fatty acid profile and total oil content are not affected by postharvest storage [32], only the results corresponding to harvest date (0 d) are reported.

2.5. Polar Metabolite Profile Analysis

The methodology of Hatoum et al. [33] was used for polar metabolite analysis. One hundred mg of frozen avocado powder was weighed, 500 µL of cold methanol was added, in addition to 20 µL of internal standard (Phenyl β-D-glucopyranoside (2910 ng μL^{-1}) in methanol), and the mixture was incubated for 20 min at 70 °C and subsequently centrifuged at 17,000× *g* for 20 min. The chromatographic conditions for the analysis corresponded to injector at 220 °C, interface at 280 °C, source at 230 °C, quadrupole at 150 °C and scanning at 50–600 *m*/*z*, with 2.66 scan/s cycles. In addition, helium was used as the carrier gas at a flow of 1 mL/min. For sugars, the split 1:150 was used at 120 °C for 1 min, and for acids, injection was carried out splitless at 50 °C for 1 min. The different metabolites were identified using the mass spectrum and the retention time of the internal standards as described by Fuentealba et al. [34]. Additionally, the NIST 2014 library was used to identify each metabolite analyzed. Metabolites were expressed in relative concentrations based on a pool of samples injected.

2.6. Statistical Analysis

A completely randomized experimental design was used, evaluating the results with an analysis of variance (ANOVA) and using a significance of 5%. For the quality parameters evaluated, different ANOVA were performed; for the monitoring of loss of firmness during storage, a one-way ANOVA was performed to evaluate if there were significant differences between harvests (early and middle) and seasons (2016/2017 and 2017/2018). Subsequently, a three-way ANOVA was performed considering, as factors, rootstocks "Duke 7" and "Mexicola", storage conditions (regulated air (RA, 5 °C) and in controlled atmosphere (CA, 4 kPa of O_2 and 6 kPa of CO_2 at 5 °C) and storage days (8 d, 21 d, 30 d and 55 d). For the other quality parameters such as color and vascular and flesh browning, a two-way ANOVA was performed. A one-way ANOVA was performed to analyze the fatty acid profile.

Ripening heterogeneity was analyzed using Minitab®17 software, and a Tukey test was performed to evaluate whether there was an effect of season, harvest, rootstock, storage type and storage time. For polar metabolites, a PLS-DA (Partial Least Squares Discriminant Analysis) was performed for each of the storage conditions (RA and CA) using the MetaboAnalyst statistical platform (https://www.metaboanalyst.ca/ (accessed on 17 May 2022)).

3. Results and Discussion

3.1. Evaluation of Global Quality Parameters and Postharvest Performance

Due to the high heterogeneity that Hass avocado fruits present in different quality attributes, it was relevant to analyze firmness data from seasons (2016/2017 and 2017/2018) and harvests (early and mid) in a general way, without considering factors, as mentioned in the materials and methods section. As can be seen in Figure 1, the one-way ANOVA showed no significant differences.

Subsequently, the data were analyzed using a three-way ANOVA (considering as factors the two rootstocks, the two storage conditions and days of storage). This statistical analysis revealed that the significant factors (p-value < 0.0001) corresponded to the storage conditions (regular air and controlled atmosphere) and days of storage (Figure 1).

As mentioned in previous research, the firmness and exocarp color of Hass avocado are the main quality attributes that characterize its ripening stage and consequently the acceptability of the batch in distribution centers or retail stores [35]. At harvest, Hass avocado mesocarp firmness usually ranges between 80 and 120 N, determined as a non-destructive compression force, which decreases moderately during regular air cold storage, and the rate only significantly increases during the shelf-life period, resulting in firmness levels below 5 N [36,37]. Results revealed an important significant difference (p-value < 0.0001) among storage days, with firmness decreasing from 8 to 55 d. The loss of firmness results from the

activity of the enzymes involved in cell wall remodeling [38], activity that is affected, as is the rate of softening, by the temperature and atmospheric conditions of storage [5].

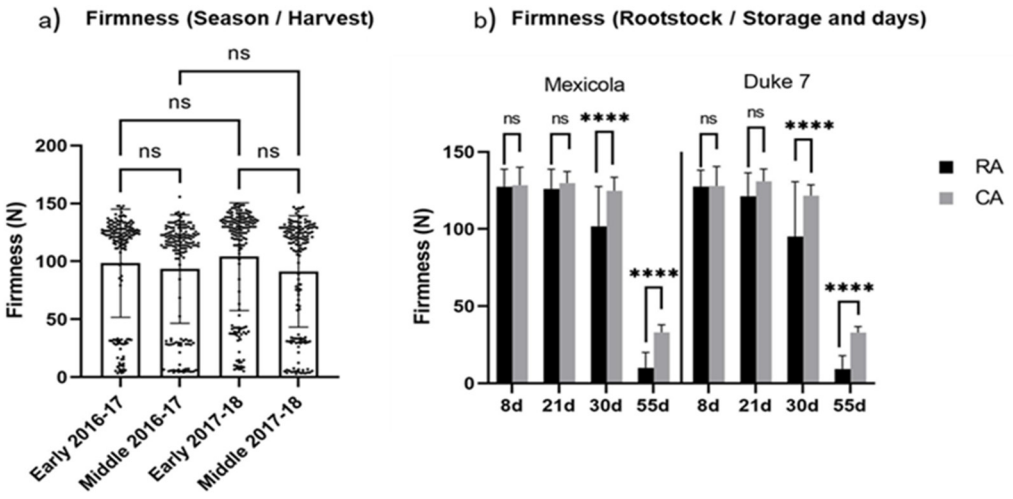

Figure 1. (**a**) Average firmness monitoring of Hass avocado fruit from "Mexicola" and "Duke 7" rootstocks from early (23–26% dry matter) and middle (>26–30% dry matter) harvests during two seasons, 2016/2017 and 2017/2018, and stored up to 55 d in regular air (RA, 5 °C) and controlled atmosphere (CA, 4 kPa O_2 and 6 kPa CO_2 at 5 °C). A two-way ANOVA was performed to evaluate whether there were significant differences between harvests and seasons. Significant differences are represented by an asterisk (ns, not significant). (**b**) Average firmness monitoring of Hass avocado fruit from "Mexicola" and "Duke 7" rootstocks stored up to 55 d in regular air (RA, 5 °C) and in controlled atmosphere (CA, 4 kPa O_2 and 6 kPa CO_2 at 5 °C). A three-way ANOVA was performed considering rootstock, storage conditions and days of storage as factors. Significant differences are represented with an asterisk (****, *p*-value < 0.0001; ns, not significant).

No significant difference between the two rootstocks evaluated ("Mexicola" and "Duke 7") with respect to firmness were observed. CA storage (4 kPa O_2 and 6 kPa CO_2 at 5 °C) largely influenced the firmness retention capacity of the Hass avocado batches. Similar results were reported by Hernández et al. [5] and Olivares et al. [39].

To date, as far as we know, there are no studies on the impact of different rootstocks related to the postharvest performance of the Hass avocado fruit, including loss of firmness during prolonged storage. The studies that have been carried out are related to the evaluation of different propagation methods (sexual and asexual) for Hass avocado rootstocks, which have revealed morphological differences in the root system that could be translated into clonal rootstocks having a vascular system with greater transport capacity [40], but without presenting any correlation with the retention of fruit firmness. In addition, there are studies that have evaluated the effect of the rootstock on Hass avocado fruit quality and its capacity to overcome pathological problems [24,26,41]. These previous studies did not include the evaluation of the firmness retention capacity of the different rootstocks during prolonged storage, simulating internal and distant markets.

Other important quality parameters, such as color, vascular browning and flesh browning, were separately analyzed at the exit of storage (RA and CA) at 30 and 55 days by means of a two-way ANOVA. For each of the quality parameters, it was first evaluated if there were significant differences between the harvest and the season, because in almost all cases (except flesh browning at 55 days with a very low significance) there were no significant differences (Supplementary Figure S1). The rootstocks were then analyzed, considering the type of storage. For the three quality parameters evaluated at 30 days of storage, for both regular air and controlled atmosphere, there were no significant differences. Different results were obtained for 55 days of storage, where significant differences between storage types and rootstocks were observed (Figure 2).

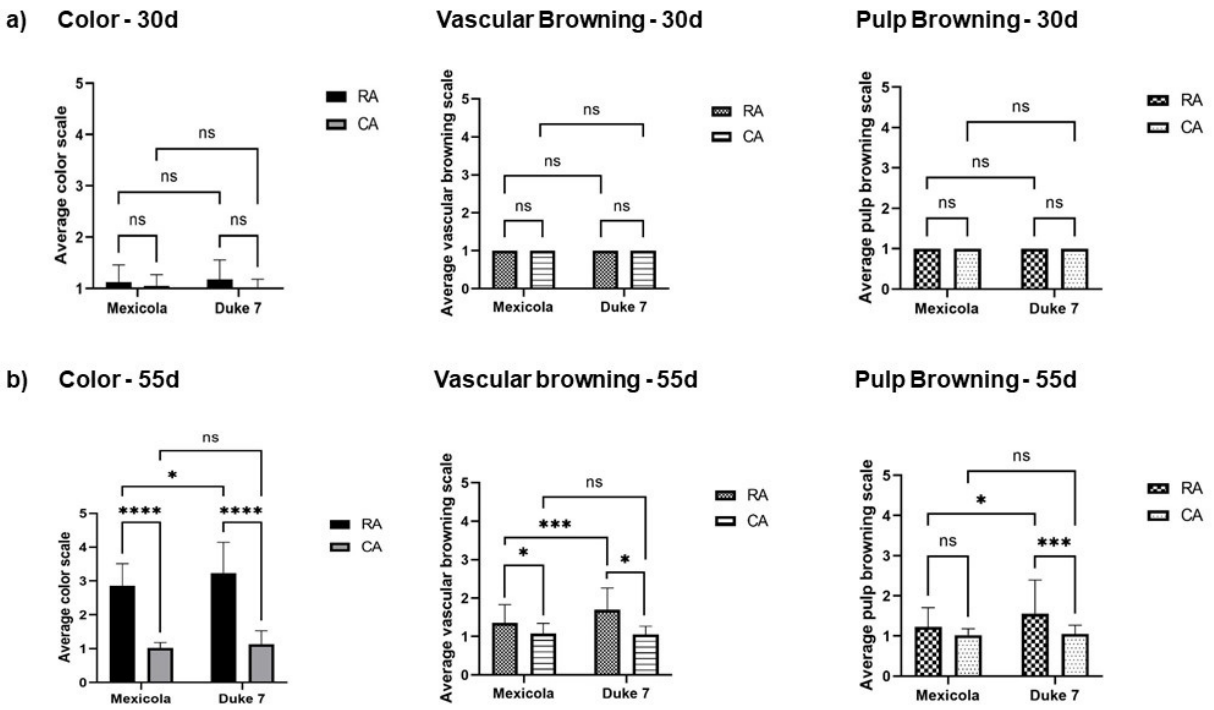

Figure 2. (**a**,**b**) Color and vascular and pulp browning for Hass avocado fruit from "Mexicola" and "Duke 7" rootstocks stored up to 30 d (**a**) and 55 d (**b**) in regular air (RA, 5 °C) and controlled atmosphere (CA, 4 kPa O_2 and 6 kPa CO_2 at 5 °C). A two-way ANOVA was performed, considering rootstock and storage conditions as factors. Significant differences are represented with an asterisk (*, *p*-value < 0.05; ***, *p*-value < 0.001; ****, *p*-value < 0.0001; ns, not significant).

Previous studies have reported that Hass avocado batches stored in regular air at 5 °C for prolonged periods have a higher incidence of internal and external physiological disorders [39]. Our results revealed similar behavior because, for the three quality parameters evaluated, the fruit stored in regular air, regardless of the rootstock evaluated, presented a higher incidence of browning and color turning at the exit of storage at 55 days (Figure 2). Previous studies by Marques and Hoffman [26], Hoffman et al. [42] and Willingham et al. [41] reported that the rootstock used can affect fruit quality in terms of the minerals present in the pulp, which could affect the incidence of vascular browning, diffuse discoloration of the pulp and fruit rotting after cold storage, with clonal rootstock being more susceptible than seed, but without a clear explanation on the mechanisms involved. Our results revealed similar behavior, but with a prolonged storage (55 days) in regular air at 5 °C. Hass fruit coming from "Duke 7" rootstock were more sensitive to browning and presented higher incidence of color turning problems than Hass avocado fruit from "Mexicola" rootstock. This situation only occurred in RA storage, because in CA storage at 5 °C no differences were observed between rootstocks in terms of the three quality parameters evaluated. These results revealed the effectiveness of CA storage for distant markets in terms of firmness retention, color turning and less development of physiological disorders [5].

Regarding color, our results revealed that, for both evaluated rootstocks at the exit of storage (30 and 55 days) in RA at 5 °C, Hass avocado fruit showed a higher degree of color change than those stored in CA (4 kPa O_2 and 6 kPa CO_2 at 5 °C), but only at 55 days of storage were the differences between rootstocks and storage condition significant (Figure 2). Similar results were reported by Arancibia-Guerra et al. [6], who revealed that fruit stored in regular air at 5 °C for 40 days showed a greater color change than those stored in controlled atmosphere, being more evident in fruit with a dry matter range of >23–26%. Despite the importance of color as a quality parameter, to date there are no

studies that have evaluated the effect of the rootstock on the color of Hass avocado fruit after prolonged storage. Instead, our results suggest that "Duke 7" rootstock, of clonal origin, could have overexpressed mechanisms involved in color turning, so it would be interesting to investigate these mechanisms in future research.

More recent studies in other species have shown that the rootstock has a relevant influence on fruit quality; for example, in "Flame Seedless" grapes, the Paulson rootstock had a positive impact on the improvement of the physical and chemical quality attributes of the bunches during the useful life as it increased the amount of ascorbic acid at harvest and minimized the incidence of darkening of the rachis by preserving a higher content of phenolic compounds [43]. There are other examples, such as in apple cv. Fuji, where it was shown that the rootstock impacted ethylene evolution, respiration, yield, fruit weight and other quality attributes [44]. In addition, it has been reported in Persian lemon that the "Flying dragon" rootstock gave the fruit a darker and more intense green color and higher values of total soluble solids, parameters that are directly related to better fruit quality [45]. All these previously mentioned studies and our results provide evidence on the relevance for the Hass avocado industry to investigate the effect of rootstocks, both of clonal and seed origin, on more complex quality attributes of Hass avocado fruit. Although it has been reported that rootstocks of clonal origin provide desired characteristics such as higher orchard productivity, these have a significantly higher cost [46]. The current complete study included the evaluation of postharvest performance of the fruit from both rootstocks, simulating different internal and export conditions, and revealed that at least for these two evaluated rootstocks ("Duke 7" and "Mexicola") in terms of fruit quality, the clonal "Duke 7" did not outperform "Mexicola".

Finally, ripening heterogeneity at edible ripeness was assessed for the fruit batches of the two rootstocks and the two storage conditions (RA and CA). Ripening heterogeneity is a quality problem that represents serious logistical problems in the avocado supply chain for importing countries as it translates into higher labor costs due to reclassification upon arrival of the fruit and significant inconsistency in quality [47]. Our results revealed that only the early season with 30 d storage presented a significant difference between the evaluated rootstocks independent of storage conditions (Figure 3). This is the first research that has evaluated differences in terms of ripening heterogeneity between a seed rootstock vs. a clonal one that is supposed to present less heterogeneity. Our results revealed that the clonal rootstock "Duke 7" batches showed significantly lower ripening heterogeneity than the seed "Mexicola" batches, not related to the capacity to accumulate dry matter that each rootstock has, because previous studies have shown that there is no correlation between the percentage of dry matter and the reduction of heterogeneity [28,47], nor with the rate of softening of the rootstocks because no significant differences were evidenced in our results (Figure 2). Therefore, it would be interesting in future research to investigate mechanisms expressed in the clonal rootstock that could be influencing the synchronization of ripening after 30 days of storage.

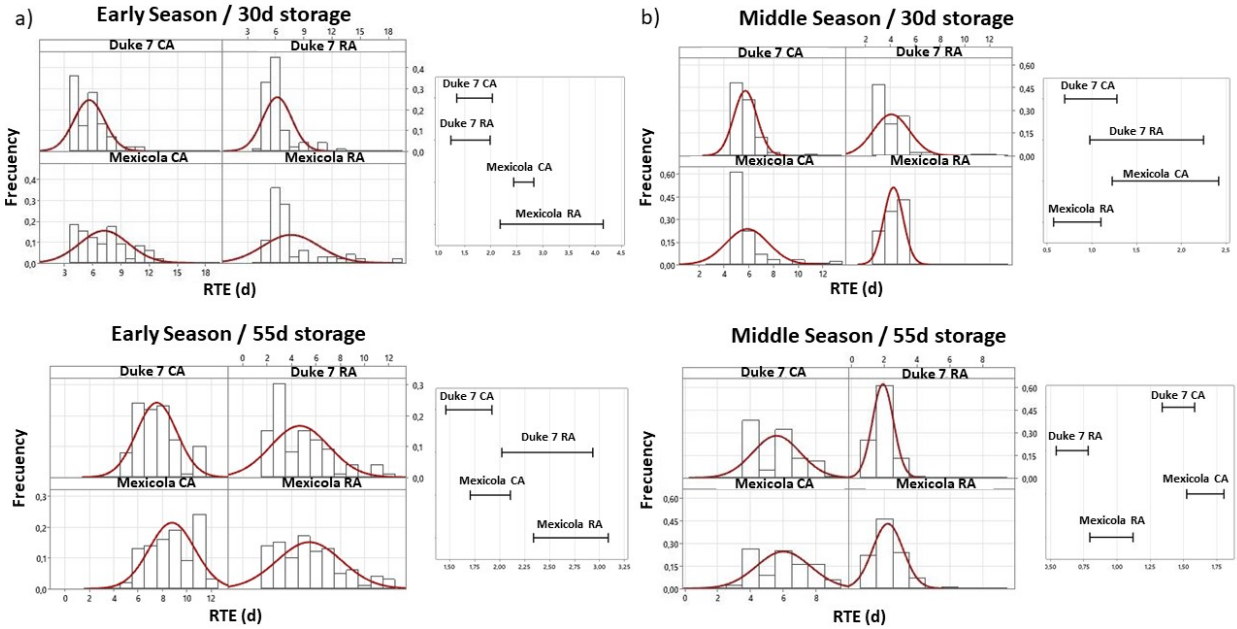

Figure 3. Evaluation of ripening heterogeneity: (**a**) early harvest fruit and (**b**) middle-harvest fruit. The treatments corresponded to: "Duke 7" and "Mexicola" rootstocks stored for 30 d and 55 d in regular air (RA, at 5 °C) and controlled atmosphere (CA, 4 kPa of O_2 and 6 kPa of CO_2 at 5 °C). Significant differences were evaluated at $p < 0.05$ using the methods of Brown and Forsythe [48] and Banga and Fox [49] and visualized as described by [50]. Histograms show the percentage of avocados reaching each RTE (ready to eat). Bars show how many avocados ripened at each RTE (d). Figures represent the confidence intervals of the standard deviation of the RTE (d).

With respect to the other evaluations of harvest, days of storage and type of storage, our results revealed that the significant differences are related to the type of storage (RA vs. CA) rather than to the evaluated rootstock (Figure 3). RA reduced ripening heterogeneity as previously reported by Defilippi et al. [51] and Fuentealba et al. [28], where fruit stored in regular air at 5 °C were more homogeneous at edible ripeness than those stored in controlled atmosphere (4 kPa O_2 and 6 kPa CO_2 at 5 °C) conditions. However, we have shown above that to reach distant markets with excellent quality in terms of firmness, color and incidence of physiological and pathological disorders, CA transport is necessary. The lower synchronization of fruit stored in controlled atmosphere may be due to the fact that this technology seeks to extend the shelf life and thus delay ripening. This technology is based on reducing oxygen levels and increasing carbon dioxide during storage, which leads to a reduction in the enzymatic activity of 1-aminocylpropane carboxylic acid oxidase (ACO), which catalyzes a key step in ethylene production, leading to quality benefits such as reduced softening rate, physiological disorders and color turning, among others [39], but it is not able to synchronize all fruit batches to trigger all the changes involved in edible ripeness. However, at destination markets, industry tends to cold store the fruit for several days to help reduce the ripening heterogeneity prior to forced ripening.

3.2. Evaluation of the Metabolic Fruit Profile

3.2.1. Analysis of the Fatty Acid Profile

The fatty acid profile obtained by GC-FID (Supplementary Figure S2) was analyzed by a one-way ANOVA, in which no significant differences were observed (p-value < 0.05) between the rootstocks evaluated ("Mexicola" and "Duke 7"). The fatty acid profile obtained confirmed oleic acid as the predominant fatty acid in Hass avocado, values very similar to those reported by Ferreira et al. [52] and Hernández et al. [47] for Chilean Hass avocados. Although no significant differences were observed in the fatty acid profile of the rootstocks evaluated (Figure 4), clonal rootstock fruit showed a trend of lower fatty acid content.

According to the study by Ferreira et al. [52], the fatty acid content is influenced by different climatic and nutritional factors; with respect to the nutritional ones, it was observed that high levels of N and Mg in fruit mesocarp at harvest were related to lower 16-carbon fatty acid content, but in that work different rootstocks were not evaluated. In the study of Gudenschwager et al. [53], chilling injury translated into flesh browning was related to genes related to lipid metabolism. Our results showed that fruit from the clonal rootstock are more susceptible to browning than fruit from the seed rootstock, which in turn have a tendency to have a lower fatty acid content. Although to date there is no research that has evaluated the differences in the fatty acid profile or content between clonal and seed rootstocks, the results of this research showed some differences in the mechanisms involved in fatty acid metabolism between Hass avocado fruit from "Mexicola" and "Duke 7" rootstocks.

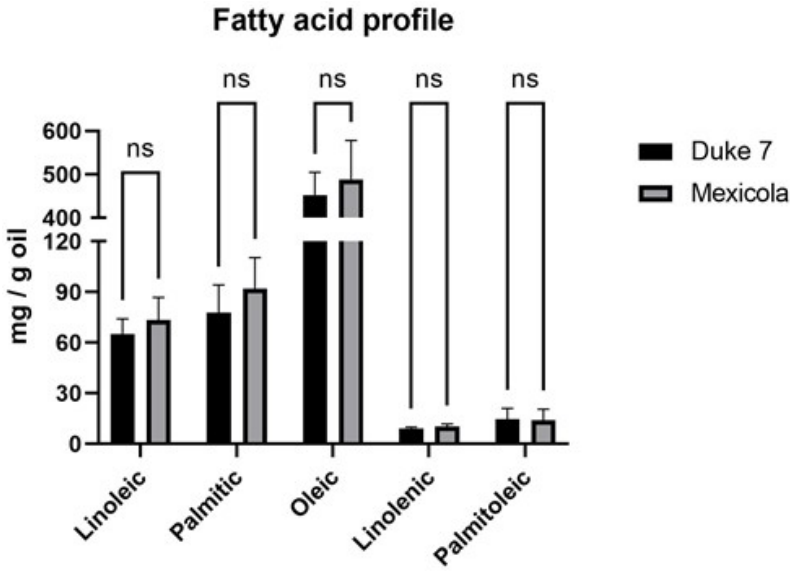

Figure 4. Fatty acid profile of Hass avocado fruit from "Mexicola" and "Duke 7" rootstocks corresponding to early harvest of the 2016/2017 season. A one-way ANOVA was performed. Significant differences were assessed at a p-value < 0.01 (ns, not significant).

3.2.2. Analysis of Polar Metabolite Profiles

First, a PLS-DA was performed considering the days of storage (8, 21 and 30), the evaluated rootstocks ("Mexicola" and "Duke 7") and the storage conditions (RA and CA). The analysis was not able to separate the groups (Supplementary Figure S3) with a percentage of variance explanation of 9% and 9.9% of components 1 and 2, respectively. In order to be able to evaluate if there are differences between the rootstocks at the polar metabolite level and if these differences are influenced by the type of storage, two separate PLS-DA by storage type (RA and CA) were performed. In both PLS-DA, it can be observed that the groups by rootstock are better separated with a higher percentage of explained variance. For instance, for the PLS-DA of CA, 19.5%, 7.8% and 7.7% for components 1, 2 and 3, respectively, were explained, whereas for RA, 11.3%, 10.8% and 10.9% for the three components were explained (Figure 5).

In order to visualize the most abundant metabolites related to each rootstock and storage condition, a heatmap was made using the VIP variables (metabolites) obtained from each of the PLS-DA performed by storage condition (RA and CA). Figure 6 shows that the metabolites with the highest abundance for both CA and RA are 6- and 7-carbon sugars, amino acids, organic acids and fatty acids, which in general terms are repeated in the two storage conditions but show contrasting behavior among the evaluated rootstocks. "Duke 7", the clonal rootstock, presented a higher mannoheptulose; the opposite situation for the seeded rootstock "Mexicola" was encountered. It has been widely reported that mannoheptulose and perseitol are the main mobile and readily available sugars for avocado growth and development, with mannoheptulose being a precursor of perseitol [54–59].

Although the importance of mannoheptulose for avocado is known, not all of its functions are yet elucidated, but it has been reported to participate in the regulation of carbon flux and protection against oxidative damage [57,58]. The results obtained in this study showed that fruit from the "Duke 7" rootstock stored in RA presented a greater and more significant difference in the incidence of browning than fruit from the Mexicola rootstock (Figure 2), which could give us indications that the clonal rootstock preserves its quality characteristics much better under controlled atmosphere storage. Among the most important metabolites involved in the discrimination (VIP metabolites), 6-carbon sugars such as sucrose, glucose, fructose, galactose and mannitol, among others, were found. In a previous study by Liu et al. [56], it was reported that in the initial stages of avocado development the predominant sugars are glucose, fructose, D-mannoheptulose and perseitol, but as development progresses the levels of fructose and glucose decrease, because the 6-carbon sugars are used during the ripening process. There is a clear difference in the abundance of these sugars between the fruit of "Duke 7" and "Mexicola" rootstocks, which could affect the quality of the fruit after prolonged storage and its postharvest performance.

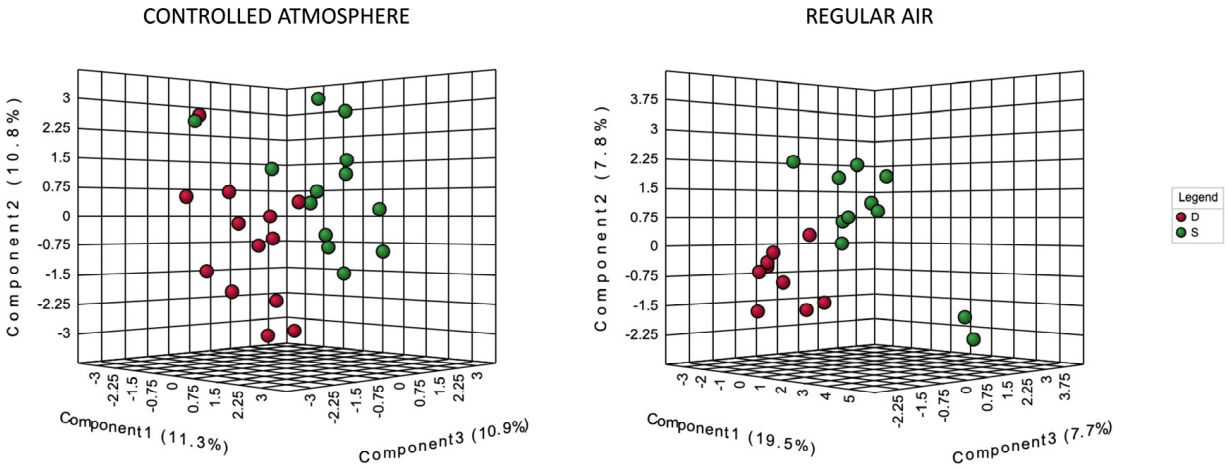

Figure 5. Partial Least Squares Discriminant Analysis (PLS−DA) score plot showing the first three components for the two storage conditions: regular air (RA, at 5 °C) and controlled atmosphere (CA, 4 kPa O_2 and 6 kPa CO_2 at 5 °C). The colored circles represent the rootstocks evaluated, "Duke 7" and "Mexicola".

Another group of VIP metabolites observed in Figure 6 are amino acids; despite the relevance of these metabolites in fruit quality and flavor, to date there are very few studies on the behavior of the different amino acids during avocado fruit growth, development and ripening. According to Pedreschi et al. [3], amino acid metabolism provides precursors for protein synthesis, for respiration processes and for a number of specialized metabolites. Therefore, fruit with a higher content of amino acids might provide substrates for the respiration process without a detrimental effect on quality. Ripening heterogeneity is a relevant quality parameter for the Hass avocado industry. Related studies have reported that amino acid metabolism, protein folding, transport and translation play an important role [4,28,37], so based on the profile of the VIP metabolites obtained (Figure 6) with the ripening behavior of the rootstocks (Figure 3), the amino acid content could be related to the ripening homogeneity of these fruit.

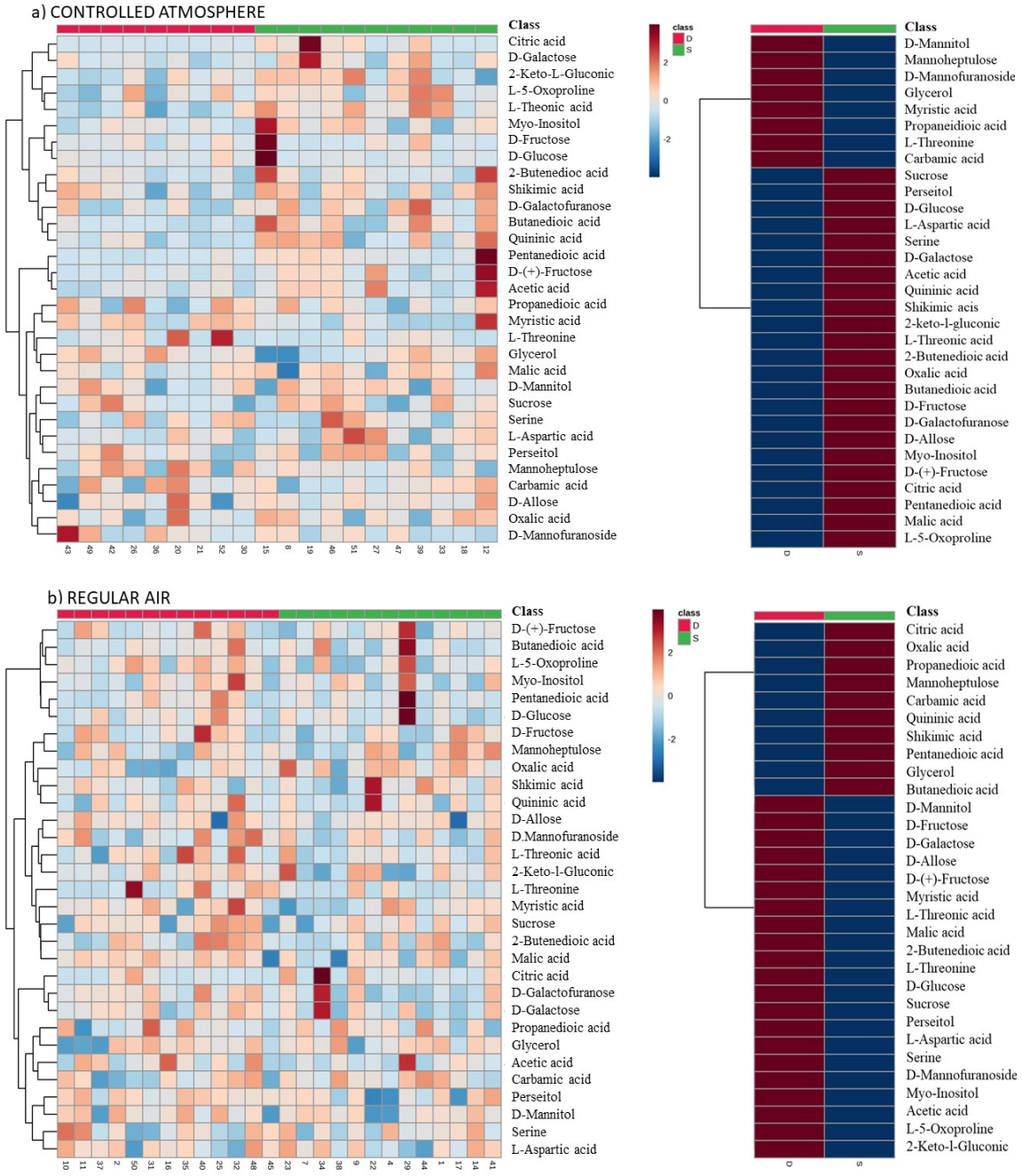

Figure 6. Heat map based on hierarchical clustering and significant VIP metabolites. (**a**) corresponds to storage in controlled atmosphere (CA). (**b**) corresponds to storage in regular air (RA). The lower axis corresponds to samples and biological replicates. The right Y axis corresponds to the very important metabolites (VIP). A heat map is observed for each of the replicates of each rootstock and another heat map using the average of the samples.

4. Conclusions

This is the first work to characterize two rootstocks of different reproductive origin, "Mexicola" seed and "Duke 7" clonal, based on different postharvest quality parameters. Although both rootstocks are widely used in the Chilean Hass avocado industry, there are very few reports with a solid scientific basis that provide information on the differences, and therefore the benefits, with respect to fruit quality that each rootstock can provide. In this work, it was observed that firmness retention, a very important parameter for reaching export destinations, did not show significant differences between rootstocks, but

rather the differences were observed with respect to the storage condition. Differences between "Duke 7" and "Mexicola" rootstocks in color and vascular and pulp browning were observed. For very prolonged storage (55 d), either in RA or CA, the clonal rootstock presented higher quality complications, so it would be less recommended for more distant export destinations, such as Asia.

Based on the importance for the Chilean industry to arrive at destination market with homogenous batches, there were no clear differences in ripening heterogeneity with respect to the rootstock, but rather due to the storage condition.

No differences in the fatty acid profile and content were found between avocado cv. Hass from both rootstocks. Finally, the polar metabolite analysis revealed differences between rootstocks; however, the abundance of the important metabolites largely depended on the storage conditions (RA or CA). This work represents the first complete effort to characterize differences between fruit from "Duke 7" and "Mexicola" rootstocks considering different important quality parameters in simulated internal and distant market scenarios, providing relevant information for the avocado supply chain.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020184/s1, Figure S1: Color, vascular and pulp browning for Hass avocado fruit from "Mexicola" and "Duke 7" rootstocks of early (23–26% dry matter) and middle (>26–30% dry matter) harvests of the 2016/2017 and 2017/2018 seasons stored up to 30 d (**a**) and 55 d (**b**). A two-way ANOVA was performed, considering harvest and seasons factors to evaluate if there were significant differences between harvest and seasons. Figure S2: Chromatograms of fatty acid methyl esters for Hass avocado from "Mexicola" (black color) and "Duke 7" (red color) rootstocks. Figure S3: Partial Least Squares Discriminant Analysis (PLS-DA) score plot showing the first three components for days of storage (8, 21 and 30), rootstocks evaluated ("Mexicola" and "Duke 7") and storage conditions (RA and CA).

Author Contributions: Conceptualization, R.P.; methodology, V.M., C.F. and I.H.; formal analysis, I.H.; research, R.P., V.M. and I.H.; writing—preparation of the original draft, R.P. and I.H.; writing—revising and editing, B.G.D., C.F. and J.E.A.; supervising, R.P.; project administration, R.P.; obtaining funding, R.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been funded by Fondecyt Grants N° 1220223 and N° 11140052 and Fondequip EQM140074 from Agencia Nacional de Investigación y Desarrollo (ANID, Chile).

Data Availability Statement: All relevant data are shown in the figures.

Acknowledgments: The authors acknowledge the Experimental Station La Palma of Pontificia Universidad Católica de Valparaíso for providing the plant and the fruit material.

Conflicts of Interest: The authors declare no conflict of interest.

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