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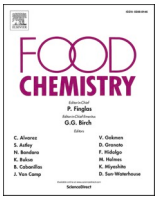

Prolonged on-tree maturation *vs.* cold storage of *Hass* avocado fruit: Changes in metabolites of bioactive interest at edible ripeness

Irene Serrano-García [a], Elena Hurtado-Fernández [b], José Jorge Gonzalez-Fernandez [c], José Ignacio Hormaza [c], Romina Pedreschi [d], Patricia Reboredo-Rodríguez [e], María Figueiredo-González [e], Lucía Olmo-García [a,*], Alegría Carrasco-Pancorbo [a]

[a] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Ave. Fuentenueva s/n, 18071 Granada, Spain
[b] Institute of General Organic Chemistry (IQOG-CSIC), Spanish National Research Council, C/ Juan de la Cierva 3, 28006 Madrid, Spain
[c] Institute for Mediterranean and Subtropical Horticulture (IHSM La Mayora-UMA-CSIC), 29750, Algarrobo-Costa, Málaga, Spain
[d] Pontificia Universidad Católica de Valparaíso, Facultad de Ciencias Agronómicas y de los Alimentos, Escuela de Agronomía, Calle San Francisco S/N, La Palma, Quillota 2260000, Chile
[e] Food and Health Omics. Department of Analytical and Food Chemistry, Faculty of Science, University of Vigo – Ourense Campus, 32004 Ourense, Spain

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ABSTRACT

When the recipient of the product is relatively distant from the production area, it is necessary to use cold storage and controlled humidity to transport the avocado fruits. One of the main advantages of local avocado consumption lies on the possibility of prolonging on-tree maturation; this could foreseeably modify the metabolic profile of the fruit that reaches the consumer. In this work, the effect of prolonged on tree maturation (during different time intervals) on the final composition of avocado fruit (at edible ripeness) was evaluated and compared with the impact of the same periods after prolonged cold storage. The quantitative evolution of nine bioactive metabolites (7 phenolic compounds, pantothenic and abscisic acids) over 40 days (10-days intervals) was studied by using a solid–liquid extraction protocol and a LC-MS methodology. The results were discussed both considering the quantitative evolution of each individual compound and the sum of all of them.

1. Introduction

The avocado (*Persea americana* Mill.) is native to Central America and Mexico, where it was domesticated and cultivated in pre-Columbian times. Traditionally, avocado genotypes have been divided into three ecological races or subspecies (West Indian, Mexican and Guatemalan) mainly related to their ecological and climatic preferences and botanical characteristics. Thus, the Mexican and Guatemalan subspecies are originated from highland regions in Central America (adapted to colder conditions) whereas the West Indian subspecies is originated from lowland regions with tropical climates (adapted to warmer conditions). The subspecies also differ in the fruit rate maturity and oil percentage (Chen, Morrell, Ashworth, De La Cruz, & Clegg, 2009). The most common avocado variety in the international markets is *Hass* (a Guatemalan × Mexican hybrid), originated as a chance seedling in California in the 1920s and that shows a buttery flavour and a pear shape that changes from deep green to dark purplish black at the edible ripeness stage. In terms of fatty acids, avocado's average nutritional profile consists mainly of monounsaturated (≈73%), saturated (≈15%) and polyunsaturated fatty acids (≈12%), that are associated with a lower risk of cardiovascular diseases (Dreher & Davenport, 2013; Ramos-Aguilar et al., 2019).

Avocado relevance in the international markets has increased exponentially in recent years and, in 2019, avocado world production was over 7 million tons. Most of the production is concentrated in a few countries (Mexico, Dominican Republic, Peru, Indonesia, Colombia, Brazil), Mexico being the largest world producer with about 32% of the total world production (more than 2 million tons) (FAO, 2021). South America stands out in the global market with 23% of total world production in 2019 with the *Hass* variety as the main cultivar for the export market, mainly to the USA and Europe. European production represents only 1.5% of the world market share, with Spain standing out as the main producing country in Europe with more than 90% of the European avocado production (FAO, 2021). Spanish production is destined mainly

* Corresponding author.
E-mail address: luciaolmo@ugr.es (L. Olmo-García).

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to France, the Netherlands, Germany and the United Kingdom, but it represents less than 10% of the total avocado consumption in Europe, so fruit need to be imported from other countries, primarily Chile and Peru to satisfy the demand. These countries are quite distant from their destination markets, with travel times of up to 55 days, so ensuring that the fruit arrives at its final destination with the highest possible quality involves the use of cold storage and controlled atmosphere (Hernández et al., 2021). In contrast, for domestic and continental exports, land transport and cold storage (approximately for 30 days) at controlled temperature (4–5 °C) and humidity (≈90%) is adequate.

On the other hand, the increasing popularity of avocado worldwide has forced to improve the distribution chain and harvest management of the fruit. The complexity of avocado fruit physiology is undeniable; in fact, the optimal strategy for identifying physiological maturity, which is not accompanied by external changes, is still unclear. Some of the most commonly used maturity indices to date are the oil concentration, fruit firmness, growth rate or dry matter (DM), which is related to oil content (Ncama et al., 2018). Portable near-infrared spectroscopy, characterized for being a non-destructive determination, is becoming a useful system to determine fruit DM, although the system has to be optimized for each variety of interest (Blakey, 2016; Burdon et al., 2015). Avocado fruit development can be divided in two different, easily distinguishable processes: fruit maturation, which is the process of growing taking place while in the tree; and post-harvest ripening, comprising the softening of the mesocarp and improvement of organoleptic properties taking place only after the detachment of the fruit (Lewis, 1978; Rodríguez-López, Hernández-Brenes, Treviño, & Díaz de la Garza, 2017; Schroeder, 1958).

External pre-harvest (e.g., light intensity and temperature), harvest (maturity stage or harvest stage) and postharvest (i.e., processing, handling, and storage) factors affect the final fruit composition. Carotenoids, sterols, phenolic compounds, carbohydrates, amino acids, proteins, vitamins, tannins, phytohormones and terpenoids, among others, have been determined in avocado, some of them predominantly in the mesocarp and others in the seed and peel (Bhuyan et al., 2019). The composition of a food and its quality is a well-recognized pairing. In this regard, the advent of sophisticated analytical techniques has opened up new frontiers and possibilities for scientists to dig deeper into the food composition. Qualitative and quantitative determinations of food metabolome offer insights into the content of the food analytes and details about some other valuable additional features (quality, authenticity, safety, health benefits, plant/fruit physiology…).

Many authors have reported that some of the bioactive compounds found in avocado participate in the mitigation of oxidative stress and inflammatory processes, reduce platelet aggregation, regulate lipid and carbohydrate metabolism, exhibit anti-cancer and neuroprotective effects or help maintaining memory and brain health (Alkhalaf, Alansari, Ibrahim, & ELhalwagy, 2019; Ortega-Arellano, Jimenez-Del-Rio, & Velez-Pardo, 2019; Ramos-Aguilar et al., 2019; Salazar-López et al., 2020).

Maturation and ripening are very complex processes and different analytical platforms have been used to study the metabolic profile and composition changes in avocado over the harvest season, during ripening, and after prolonged cold and controlled atmosphere storage. Table 1 gathers some interesting papers that have dealt with these topics (evolution of fruit composition over maturation and ripening), including different experimental designs, storage conditions, time intervals (dates considered), analytical platforms and determinations carried out. This table is not intended to represent an exhaustive literature review, but rather a collection of several works that exemplify different experimental designs, determinations of interest, etc. As can be deduced from the just mentioned table, the selected examples focused on how some parameters of interest (DM, oil content, total soluble proteins, etc.) as well as some specific compounds (phenolic compounds, carotenoids, tocopherols, sugars, fatty acids, etc.) evolved during the harvest season of different varieties or over ripening. Other very valuable works regarding maturation over the harvest season and/or ripening could also be cited (Arpaia, Collin, Sievert, & Obenland, 2015; Bower & Cutting, 1988; Ferreyra et al., 2012; Mpai & Sivakumar, 2020; Obenland, Collin, Sievert, Negm, & Arpaia, 2012; Wang, Zheng, Khuong, & Lovatt, 2012).

To the best of our knowledge, there is no study comparing the effect of prolonged on-tree maturation *vs*. cold storage over a 40-days period (at 10-days intervals) on the metabolic profile of *Hass* avocado fruit at the ready-to-eat stage. To fill this gap, the aim of this work was to study the evolution of nine metabolites of interest in avocado fruit stored at 4 °C for 10, 20, 30 and 40 days compared to fruit that remained on the tree during the same period of time. The monitored compounds were epicatechin, *p*-coumaric, ferulic, pantothenic and abscisic acids, and four hexoses of coumaric acid. Their quantitative evolution as well as how the permanence in the tree (prolonged on-tree maturation) or in cold storage affects the final composition of the fruit at the ready-to-eat stage will be discussed in this contribution.

2. Materials and methods

2.1. Chemicals and reagents

Methanol (MeOH) was the solvent used for sample extraction and preparation of stock solution and was supplied by Prolabo (Paris, France). Standards of pantothenic, *p*-coumaric, ferulic and abscisic acids as well as epicatechin were acquired from Sigma-Aldrich (St. Louis, MO, USA). Moreover, *o*-coumaric acid from Sigma-Aldrich was used as internal standard (IS) to control the repeatability of the applied analytical methodology. Stock solution at a concentration of 200 mg L^{-1} was first prepared by dissolving an appropriate amount of every metabolite in MeOH. Then, serial dilutions within the range from the quantification limit (LOQ) to 200 mg L^{-1} were prepared. All the samples and solutions were filtered by using a nylon syringe filter Clarinet™ of 0.22 μm from Bonna-Agela Technologies (Wilmington, DE, USA) and stored in dark flasks at −20 °C. Doubly deionized water with a conductivity of 18.2 MΩ was obtained by using a Milli-Q system (Millipore, Bedford, MA, USA). Acetic acid (AcH), used for the acidification of LC mobile phase A, was supplied by Panreac (Barcelona, Spain) and LC–MS grade acetonitrile (ACN) (phase B) by Lab-Scan (Dublin, Ireland). Mobile phases were filtered by using a nylon membrane filter 0.45 μm Nylaflo™ acquired from Pall Corporation (Ann Arbor, MI, USA). Reagents were of analytical or LC-MS grade and were used as received in the laboratory.

2.2. Samples

The samples considered in the current study were obtained from the unique avocado germplasm collection maintained at the Institute for Mediterranean and Subtropical Horticulture (IHSM-UMA-CSIC) La Mayora in Malaga (Spain). A total of 45 samples were analyzed. *Hass* avocado harvest season in Spain lasts approximately from December to May. The specific time period considered in this research started at the end of February (27th February) and ended in almost mid-April (9th April), covering the most important production months in Spain, with five time-points being evaluated. Table 2 contains information about the samples considered in this study, including details about date of collection/harvest in the orchard, date of release from cold storage, number of days in cold storage, and number of replicates in each case. At the beginning of the study, a considerable number of avocados were harvested from La Mayora orchard. Some avocados were stored directly at room temperature (simulating domestic ripening conditions) and left until they reached their ready-to-eat stage. The rest of the fruit was placed in the cold room (between 4.41 ± 0.84 °C and 93.05 ± 1.45% of humidity). After 10, 20, 30 and 40 days, two events took place: a) fruits were harvested again from the orchard and left at room temperature; and b) the samples that had been in the storage chamber for a certain period of time were removed and placed at room temperature. No fruit was processed until the optimum ripeness stage for consumption was reached. Each sample consisted of different pieces of mesocarp from 4 to

Table 1
Examples of interesting papers that have dealt with the evolution of avocado fruit composition over maturation and ripening.

Aim of study	Samples	Period of study	Storage conditions	Chemical determinations / Determined compounds	Analytical platform/s	Ref.
To evaluate if ripening stage influences the content of specific compounds and determine *in vitro* cytotoxic activity	60 fruit batch. A sub-lot of 25 fruits for each RS	4 RS (0-, 4-, 8- and 12-days post-harvest)	14 days at 15 °C	Seven phenolic compounds, carotenoids, tocopherols, phytosterols and cytotoxic activity	HPLC-DAD HPLC-FLD GC-FID	Villa-Rodriguez et al., 2020
To appraise the metabolic changes that occur in four varieties during its main harvesting seasons in Southern Spain	3 or 4 pieces of fruit for each time point	Specific period for each variety of avocado (different harvesting dates)	1 week at 4–6 °C and at room temperature until edible ripeness	Epicatechin, pantothenic, chlorogenic, *p*-coumaric, ferulic and abscisic acids	HPLC-ESI-IT MS	Hurtado-Fernández et al., 2016
To compare the phenolic profiles of six varieties of avocado at two different RS	36 independent extracts	2 RS (unripe and ripe fruits)	–	Eighteen phenolic compounds	UHPLC-HESI-Q-Orbitrap MS	Di Stefano et al., 2017
To evaluate the metabolic changes that occur in cv. *Reed* during the harvest season in Southern Spain	18 samples	9 dates over the harvest season between June and October (2011 season)	Samples were processed at edible ripeness	Ten different metabolites such as phenolic acids, flavonoids, a carbohydrate, an organic acid, a vitamin and a phytohormone	CE-MS HPLC-ESI-IT MS HPLC-ESI-QTOF MS	Contreras-Gutiérrez et al., 2013
To establish a proper fruit biopsy sampling approach for *Hass* avocado and to explain the ripening heterogeneity	One hundred avocados from the same tree from a commercial orchard	–	5 °C overnight before fruit biopsy. 5 RS (measured as loss of firmness, 0–5 hedonic scale)	Dry matter and total Ca^{2+}, non-polar compounds (fatty acids and lipid-soluble isoprenoids), polar (C_7 and C_6 sugars) and semi-polar compounds	GC-TOF MS GC-Q MS HPLC-PDA-QTOF HPLC-PDA HPLC-FLD	Pedreschi et al., 2014
To evaluate the application of UHPLC-TOF MS to study RS on avocado fruit	Fruit of 13 varieties at two different ripening degrees	2 RS (green and ready to eat)	Household conditions	Twenty different compounds (quinic acid, abscisic acid, benzoic acid, succinic acid, etc)	UHPLC-UV/ESI-TOF MS	Hurtado-Fernández et al., 2011
To compare the effect of different pre-harvest conditions on the main bioactive compounds changes during ripening of imported avocado cv. *Hass* fruit	240 fruits from each country (Spain, Peru and Chile)	0, 1-, 2-, 4- and 7-days post-harvest in early, middle and late season	Day 0 at 5 °C overnight Day 1, 2, 4 and 7 at 18–23 °C	Perseitol, D-mannoheptulose, sucrose, fructose, glucose and five individual fatty acids	GC-FID HPLC-RID	Donetti & Terry, 2014
To determinate changes in the concentrations and relationships between sugars, total soluble proteins and oil during *Hass* fruit ripening	Export grade *Hass* avocado fruit from commercial orchards near Tzaneen and Howick (South Africa)	5 or 6 RS (2, 5-, 8-, 11-, 13- and 15-days post-harvest or very similar intervals depending on the origin of the samples)	Ripened at 21 ± 2 °C	Oil content, total soluble proteins, perseitol, D-mannoheptulose, sucrose, fructose and glucose	HPLC-RID	Blakey, Tesfay, Bertling, & Bower, 2012
To develop a method for sequential extraction and subsequent quantification of fatty acids and sugars on avocado mesocarp tissue	72 *Hass* fruits from Malaga (Spain)	3 RS (under-ripe, medium-ripe and eating-ripe)	Fruits arrived at lab 4 days after harvest. 12 °C for 9 days in 3 L jars and then removed.	Dry matter, oil content, perseitol, D-mannoheptulose, sucrose and five individual fatty acids	GC-FID HPLC-ELSD	Meyer & Terry, 2008
To determine if exposure of fruit to sunlight could vary the biochemical compounds associated with maturity	Nine fruit per canopy and per cv. *Carmen* and cv. *Hass*	Study conducted during autumn, winter and spring seasons (February to January) during 2018/9 season	Fruits were sampled at two-week intervals and kept at 25 °C for 7–10 days to allow ripening	Dry matter, oil content, perseitol, D-mannoheptulose and total C_7 sugars	HPLC-RID	Shezi, Magwaza, Tesfay, & Mditshwa, 2020
Dry matter, oil content and fatty acid composition of *Fuerte* and *Hass* fruits were examined with respect to the harvesting and post-harvest ripening period	*Fuerte* and *Hass* avocado fruits from Antalya (Turkey)	Fruits harvested in November, December, and January at one-month intervals. 3 RS (1-, 4-, and 8-days post-harvest)	Samples were kept for 8 days under ambient conditions (18–22 °C) to ripen	Dry matter, oil content and seven individual fatty acids	GC-FID	Ozdemir & Topuz, 2004)
To evaluate the effect of RS of *Hass* avocado on the content of hydrophilic and lipophilic compounds and their correlation with the antioxidant capacity	60 fruits in total (from Michoacan, Mexico). A sub-lot of 25 avocados for each RS	4 RS (0-, 4-, 8- and 12-days after arrival at lab)	14 days at 15 °C	Respiration rate, ethylene production, dry matter, oil content, total phenolic content, flavonoid content and ten individual fatty acids	GC–MS GC-TCD GC-FID Spectrophotometry	Villa-Rodríguez et al., 2011

Abbreviations used in the table in alphabetical order: CE (capillary electrophoresis); DAD (diode-array detector); ELSD (evaporative light scattering detector); ESI (electrospray ionization); FLD (Fluorescence detector); FID (flame ionization detector); GC (Gas chromatography); HESI (heated electrospray ionization); HPLC (high-performance liquid chromatography); IT (ion trap); PDA (photodiode-array detector); Q MS (quadrupole mass spectrometry); RID (refractive index detector); RS (ripening states); TCD (thermal conductivity detector); TOF MS (time of flight mass spectrometry); UHPLC (ultra-high-performance liquid chromatography); UV–VIS (ultraviolet–visible spectrophotometer).

Table 2
Details of the *Hass* avocado samples considered in this study.

	Collection date*	Cold chamber output date*	Days in cold chamber	Number of biological replicates	DM◆
t_0	27/02	–	–	5	31
$t_{1\ cold\ stored}$	27/02	08/03	10	5	
$t_{1\ on\text{-}tree}$	08/03	–	–	5	32
$t_{2\ cold\ stored}$	27/02	19/03	20	5	
$t_{2\ on\text{-}tree}$	19/03	–	–	5	32
$t_{3\ cold\ stored}$	27/02	29/03	30	5	
$t_{3\ on\text{-}tree}$	29/03	–	–	5	33
$t_{4\ cold\ stored}$	27/02	09/04	40	5	
$t_{4\ on\text{-}tree}$	09/04	–	–	5	34

*Dates are indicated as follows: "day of the month/month".
◆ The found DM values can be considered to be normal for what is usually found in *Hass* avocados in Spain in these dates of the harvest season. SD of DM measurements were close to 1 approx.

5 avocado fruits. Each time-point in turn, as can be seen in the table, was composed of five different samples (five replicates). Fruits were peeled, chopped, lyophilized, crushed, homogenized and frozen at −20 °C.

DM was evaluated according to the AOAC 920.151 method (AOAC, 2016) as soon as the fruit were detached from the tree.

2.3. Extraction procedure

The applied sample extraction procedure was the one previously described by Hurtado-Fernández et al. (2016). Sample extracts were prepared by mixing 0.5 g of frozen, dried and homogenized sample with 40 mL of pure MeOH and the proper amount of IS to obtain 25 mg L^{-1} of it in the final extract. After 3 min of Vortex shaking, the tubes were introduced into an ultrasound bath for 30 min, with a final centrifugation step of 3 min at 5000 rpm. Once the two phases were separated, the supernatant was transferred to a flask. The solid residue was re-extracted by adding 20 mL of pure MeOH and applying the same procedure (2nd extraction cycle). Both supernatant were mixed and evaporated to dryness in a rotary evaporator. Finally, the residue was redissolved in 1 mL of pure MeOH. Two extracts were prepared for each sample.

Furthermore, to control instrument repeatability and to evaluate several parameters considered for the validation of the method, a representative quality control (QC) sample was prepared by mixing equivalent amounts of all the extracts.

2.4. Liquid Chromatography- mass spectrometry analyses

Two different LC-MS platforms were used in this study. LC-MS system with a high-resolution MS analyzer was used with qualitative purposes, whereas the LC platform coupled to a low resolution MS was used to carry out the quantitation of the analytes of interest. The instrument used to analyze the total number of avocado extracts considered within this study (with quantitative purposes) was a 1260 Infinity Agilent (Agilent Technologies, Waldbronn, Germany) equipped with a Zorbax C_{18} column (4.6 × 150 mm, 1.8 μm particle size) coupled to an Esquire 2000 Ion Trap (IT) mass spectrometer (Bruker Daltonics, Bremen, Germany) by means of an electrospray ionization (ESI) source. Some representative samples were analyzed with an Acquity UPLC™ H-Class system coupled to a QTOF SYNAPT G2 MS (Waters, Manchester, UK) through an ESI interface. The chromatographic conditions were the same in both platforms. The analytical column was set at 25 °C and analytes were eluted with 0.5% AcH in water (mobile phase A) and pure acetonitrile (mobile phase B) using a flow rate of 0.8 mL/min. The following solvent gradient was applied: 0 min, 95% A and 5% B; 20.5 min, 30% A and 70% B; 22 min, 0% A and 100% B; at 23.5 min, the system returned to initial conditions and the column was re-equilibrated for 3 min. A volume of 10 μL was injected in each case (both for extracts and pure standards).

The low resolution MS was operated in negative mode and data were acquired in Full Scan mode for a mass range from 50 to 1000 *m/z*. Optimal parameters related to ESI source were the following: the nebulizer gas (nitrogen) was set at 30 psi, and the dry gas (nitrogen) flow rate and temperature were 9 L/min and 300 °C, respectively. The capillary voltage was set at + 3200 V and the end-plate offset at −500 V. These parameters were then transferred to the ESI-QTOF MS which operated both in negative and positive modes.

Agilent ChemStation (Agilent Technologies) and Esquire Control (Bruker Daltonics) were used to operate the LC and low resolution MS systems, respectively. The high resolution MS coupling was controlled with MassLynx (Waters). DataAnalysis 4.0 software (Bruker Daltonics, Bremen, Germany) was used for MS data processing. Microsoft Excel 2019 was used for quantitative data management and for representing the data graphically. Quantitative results were reported as mg of analyte Kg^{-1} in dry basis. Analysis of variance (one-way ANOVA) was performed using Statgraphics 19 (Statgraphics Technologies, Inc., The Plains, VA, USA). The significance of the differences at 5% (p less than 0.05) level between mean values was determined using the Tukey's test.

2.5. Validation studies

Pure standard solutions (both individual pure standards and mixtures of them), the QC and spiked extracts (with known added amounts of standards) were used for the validation of the method. Linearity, precision, trueness and possible matrix effect were evaluated.

Solutions of the five pure compounds (pantothenic, *p*-coumaric, ferulic and abscisic acids as well as epicatechin) were prepared in MeOH at ten different concentration levels over the range from the quantification limit to 200 mg L^{-1} to establish external calibration curves and evaluate the linearity. Calibration curves were obtained for each standard by plotting the standard concentration as a function of the peak area and defining the least squares regression line. Each point of the calibration curve corresponded to the mean value from three independent injections (n = 3). When the pure standard of an analyte to be quantified was not commercially available, it was quantified in terms of the most similar molecule. Thus, hexoses of coumaric acid were quantified with *p*-coumaric acid external calibration curve; the other metabolites were quantified by using the equation of their own standard calibration curve. Specific calibration ranges were established for each compound.

Detection and quantification limits (LOD and LOQ) of each individual compound were calculated based on the signal/noise ratio (S/N) obtained at the lowest concentration level injected. Thus, the LOD and LOQ values were estimated by calculating the concentration that generated the S/N equal to 3 and 10, respectively.

The precision of the LC-MS method was evaluated in terms of repeatability (*intra*-day and *inter*-day) and expressed as coefficient of variation (%CV). The *intra*-day repeatability was obtained from seven injections of the QC carried out within the same sequence, whereas *inter*-day repeatability was obtained from the data from 14 injections of QC carried out on different sequences and, therefore, days. Trueness was

expressed as recovery and was estimated by analyzing the samples before and after the addition of known concentrations of pure standards and calculating the difference between the obtained results. Different standard concentration levels within the linear range (low, medium and high) were used for the spiking experiments. Finally, matrix effect was evaluated by calculating a matrix effect coefficient (Kmellár et al., 2008) that compares the slope of the standard addition calibration in the QC and the external calibration in MeOH as follows:

$$\text{Matrix effect coefficient}(\%) = (1 - \frac{slope\ of\ standard\ addition\ calbration\ line}{slope\ of\ external\ calibration\ line}) \bullet 100$$

In general, a range of ± 20% has been established to consider that matrix effect is negligible.

3. Results and discussion

3.1. Characterization of the metabolic profile of avocado mesocarp by LC-ESI-QTOF MS

A first qualitative exploration of the chromatographic profiles obtained was carried out. Avocado mesocarp is a quite complex matrix and the used LC-MS metabolic profiling approach made possible to detect a considerable number of compounds. Within the profile, a total of nine compounds were selected (see Fig. 1 Supplementary material) taking into account: i) their relative abundance in the avocado mesocarp, ii) the possible fluctuation of their concentrations during the harvest season or the ripening process, and iii) the importance of some of these compounds in previous publications. Epicatechin, *p*-coumaric, ferulic, pantothenic and abscisic acids, and four hexoses of coumaric acid were the most relevant metabolites selected to be monitored in this study.

Table 3 includes the retention time of each analyte, the detected *m/z* signals, and the peak assignment in positive and negative modes in LC-ESI-QTOF MS. MS signals for LC-ESI-IT MS in negative mode have also been reported in the table, since the quantitative analysis of the sample-set was carried out by using that coupling.

As stated, two hydroxycinnamic acids (*p*-coumaric acid and ferulic acid) and four hexoses of coumaric acid were selected to be quantified. Most hydroxycinnamic acids present in avocado are mainly conjugated with sugars or other small molecules such as quinic acid. Moreover, epicatechin (flavonoid), pantothenic acid (vitamin) and abscisic acid (phytohormone) were also appointed as analytes of interest and quantified by using their own pure standards. In elution order, the *m/z* signals detected for each metabolite were: Pantothenic acid gave a predominant MS signal at *m/z* 218.1032, epicatechin at *m/z* 289.0723, *p*-coumaric acid at *m/z* 163.0395 (together with another relevant signal at 119.0504 $[M-H-44]^-$), ferulic acid at *m/z* 193.0505 and abscisic acid at *m/z* 263.1294 (in all these cases, the *pseudo*-molecular ion, $[M-H]^-$, was the prevailing signal). In addition, coumaric acid hexose isomers I and II were tentatively identified, since their pure standards were not commercially available. Their identification was based on the following observations: a) the signals detected in TOF MS for these 2 compounds correspond to the *pseudo*-molecular ion (325.0932 and 325.0936 $[M-H]^-$, respectively), the loss of a hexose moiety (145.0292 and 145.0298 $[M-H-180\ (hexose)]^-$, for each isomer), and the typical signal of the *pseudo*-molecular ion of coumaric acid (163.0397 and 163.0403, apiece); b) they elute before *p*-coumaric acid (with a shorter retention time), which is logical, since the carbohydrate moiety confers these molecules a higher polarity; and c) other authors have previously considered the same tentative identification (Campos et al., 2020; Contreras-Gutiérrez et al., 2013; López-Cobo et al., 2016). On the other hand, the MS signals that led to the identification of coumaric acid malonyl-hexose isomers I and II correspond to the *pseudo*-molecular ion 411.0910 and 411.0921 $[M-H]^-$, the loss of a carboxylic acid moiety (367.1022 and 367.1030 $[M-H-44\ (CO_2)]^-$), the loss of an hexose (−180) and malonyl group (−86) (145.0289 and 145.0287 $[M-H-180-86\ (hexose\ and\ malonyl)]^-$) and the typical signal of the *pseudo*-molecular ion of coumaric acid (163.0388 and 163.0393). The *m/z* signal of 367.1030 has also been assigned to the molecular formula $C_{17}H_{20}O_9$ by other authors (as in our case), but they identified the substance as feruloylquinic acid (López-Cobo et al., 2016). The fact of detecting fragments typically related to *p*-coumaric acid, as well as the dimer of $C_{18}H_{20}O_{11}$ (*m/z* 823.1949), has led us to assign it the identity shown in Table 3 (coumaric acid malonyl-hexose isomer).

3.2. Analytical parameters of the LC-ESI-IT MS method

As previously stated, the applied method was validated considering linearity, LODs and LOQs, precision, trueness and matrix effect. Results were, in general, very satisfactory. The LODs ranged from 18.8 to 70.8 μg L^{-1}, whilst the LOQs fluctuated between 62.5 and 123.2 μg L^{-1}, for epicatechin and pantothenic acid, respectively. The *intra*-day repeatability did not exceed in any case the value of 7.26%, whilst the *inter*-day repeatability was always lower than 8.29% (both CV values corresponding to epicatechin). The trueness was found within the range from 97.7 to 113.0% (for pantothenic acid and epicatechin), and the matrix effect coefficients varied from −9.5 to 4.6%, for pantothenic and *p*-coumaric acids. Table 1 (supplementary material) shows the analytical parameters of the LC-MS method used for the analysis of the avocado extracts.

3.3. Quantification of metabolites of interest by LC-ESI-IT MS

Concentration values found in the present study were the mean of five biological replicates extracted twice (n = 10, 5 biological replicates × 2 analytical ones) and have been expressed in mg Kg^{-1} of dry weight (DW) with their corresponding standard deviation (Table 4).

Table 4 has been structured to give the quantitative values of each compound at the different time-points for both on-tree maturation and for avocados that were kept in cold storage. One-way ANOVA test followed by Turkey's test was applied to reveal whether there were significant differences in the concentration values among the different time-points belonging to the same strategy (on-tree or cold storage, respectively), or to determine whether there were quantitative differences at the same time-point as a consequence of the strategy considered (prolonged on-tree maturation *vs*. cold storage).

In order to evaluate in more detail the results concerning each analyte, the graphs shown in Fig. 1 were plotted. Each graph shows the evolution of each compound as the considered 10-day periods elapsed. The results for prolonged on-tree maturation are in green and those for cold storage in orange. The magnitude of the standard deviations, in some cases, is substantial, which is perfectly normal considering that each value comes from a sample composed of five biological replicates (extracted twice).

It is difficult to compare the quantitative results in absolute terms with those reported in other works, as the concentrations of these compounds are highly dependent on the avocado variety, the harvest period, the ripening index, as well as other factors listed in the

Table 3
Peak assignment of the metabolites studied in this work found in the avocado samples.

LC-ESI-QTOF MS				LC-ESI-IT MS			
ESI(+) QTOF MS[a]	**ESI(-) QTOF MS[a]**	**For experimental [M−H] -**		**Rt (min)**	**ESI(-) IT MS[a]**	**Molecular formula [M] generated**	**Assignment[c]**
		Error (ppm)[b]	**mSigma value[b]**				
220.1156 [M + H]$^+$ 242.0975 [M + Na]$^+$ 258.0636 [M + K]$^+$ 202.1050 [M + H-18]$^+$ 184.0952 [M + H-18–18]$^+$	218.1032 [M−H]$^-$ 146.0811 [M−H−28−44]$^-$ 459.1898 [2 M−H + 23]$^-$	0.4	1.8	5.6	218.0 [M−H]$^-$ 260.9 [M−H + 44]$^-$ 437.1 [2 M−H]$^-$	$C_9H_{17}NO_5$	Pantothenic acid*
349.0791 [M + Na]$^+$ 365.0582 [M + K]$^+$	325.0932 [M−H]$^-$ 163.0397 [M−H−162]$^-$ 145.0292 [M−H−162−18]$^-$ 117.0337 [M−H−162−18−28]$^-$	0.9	2.8	7.9	325.0 [M−H]$^-$ 163.0 [M−H−162]$^-$ 145.0 [M−H−162−18]$^-$	$C_{15}H_{18}O_8$	Coumaric acid hexose I
349.0793 [M + Na]$^+$ 365.0558 [M + K]$^+$	325.0936 [M−H]$^-$ 163.0403 [M−H−162]$^-$ 145.0298 [M−H−162−18]$^-$ 117.0335 [M−H−162−18−28]$^-$	1.3	4.0	8.3	325.0 [M−H]$^-$ 163.0 [M−H−162]$^-$ 145.1 [M−H−162−18]$^-$	$C_{15}H_{18}O_8$	Coumaric acid hexose II
291.0856 [M + H]$^+$ 313.0650 [M + Na]$^+$ 165.0551 [M + H-126]$^+$	289.0723 [M−H]$^-$	1.1	3.8	8.8	289.0 [M−H]$^-$	$C_{15}H_{14}O_6$	Epicatechin*
413.1051 [M + H]$^+$ 435.0866 [M + Na]$^+$ 451.0609 [M + K]$^+$ 165.0521 [M + H-162–86]$^+$ 147.0425 [M + H-86–162-18]$^+$	411.0910 [M−H]$^-$ 823.1943 [2 M−H]$^-$ 367.1022 [M−H−44]$^-$ 205.0495 [M−H−44−162]$^-$ 163.0388 [M−H−162−86]$^-$ 145.0289 [M−H−180−86]$^-$	1.6	8.2	9.4	410.9 [M−H]$^-$ 367.0 [M−H−44]$^-$ 205.0 [M−H−44−162]$^-$ 163.1 [M−H−162−86]$^-$ 145.1 [M−H−162−86−18]$^-$	$C_{18}H_{20}O_{11}$	Coumaric acid malonyl-hexose I
413.1044 [M + H]$^+$ 435.0858 [M + Na]$^+$ 451.0541 [M + K]$^+$ 165.0522 [M + H-162–86]$^+$ 147.0429 [M + H-162–86-18]$^+$	411.0921 [M−H]$^-$ 823.1949 [2 M−H]$^-$ 367.1030 [M−H−44]$^-$ 205.0500 [M−H−44−162]$^-$ 163.0393 [M−H−162−88]$^-$ 145.0287 [M−H−180−86]$^-$	1.1	17.1	9.7	411.0 [M−H]$^-$ 367.0 [M−H−44]$^-$ 205.0 [M−H−44−162]$^-$ 163.0 [M−H−162−86]$^-$ 145.0 [M−H−162−86−18]$^-$	$C_{18}H_{20}O_{11}$	Coumaric acid malonyl-hexose II
165.0477 [M + H]$^+$ 203.0017 [M + K]$^+$ 147.0433 [M + H-18]$^+$	163.0395 [M−H]$^-$ 119.0504 [M−H−44]$^-$	0.0	0.0	10.4	162.9 [M−H]$^-$ 119.1 [M−H−44]$^-$	$C_9H_8O_3$	*p*-Coumaric acid*
195.0949 [M + H]$^+$ 233.0128 [M + K]$^+$ 177.0539 [M + H-18]$^+$ 145.0271 [M + H-50]$^+$	193.0505 [M−H]$^-$ 178.0263 [M−H−15]$^-$ 134.0379 [M−H−15−44]$^-$	0.4	2.1	11.0	193.0 [M−H]$^-$ 178.0 [M−H−15]$^-$ 134.1 [M−H−15−44]$^-$	$C_{10}H_{10}O_4$	Ferulic acid*
265.1436 [M + H]$^+$ 287.1254 [M + Na]$^+$ 303.0945 [M + K]$^+$ 247.1326 [M + H-18]$^+$ 201.1273 [247–46]$^+$ 187.1117 [247–60]$^+$ 163.0749 [M + H-102]$^+$	263.1294 [M−H]$^-$ 219.1390 [M−H−44]$^-$ 153.0922 [M−H−44−66]$^-$ 549.2435 [2 M−H + 23]$^-$	1.1	4.2	13.6	263.0 [M−H]$^-$ 219.3 [M−H−44]$^-$ 153.2 [M−H−44−66]$^-$	$C_{15}H_{20}O_4$	Abscisic acid*

[a] Different *m/z* values rather than [M + H]$^+$ / [M−H]$^-$ were detected in the MS spectra; when those ions were more intense than the corresponding [M + H]$^+$ / [M−H]$^-$, they have been underlined. The mentioned different *m/z* values mainly correspond to in-source fragments (typical losses detected were −18 (H_2O), −28 (CO), −44 (CO_2), −162 (hexose)) and to sodium [M + 23]$^+$ and potassium [M + 49]$^+$ adducts, in negative and positive polarities, respectively.
[b] Values of error and mSigma did not exceed 1.6 and 17.1, apiece.
[c] (I, II) different isomers; (*) identification confirmed by comparison with authentic standards.

Table 4
Quantitative results (mg Kg^{-1}, dry weight) obtained for the determined metabolites in avocado mesocarp by using LC-ESI-IT MS.

Time (days) (t_d)	Tree	Cold storage	Tree	Cold storage	Tree	Cold storage
	p-Coumaric acid*		coumaric acid hexose I		coumaric acid hexose II	
0 (t_0)	36aΔ ± 6	36aΔ ± 6	165aΔ ± 36	165abΔ ± 36	54abΔ ± 14	54aΔ ± 14
10 (t_1)	29aΔ ± 5	40aΔ ± 4	130aΔ ± 37	232aΔ ± 98	38acΔ ± 6	54aΔ ± 16
20 (t_2)	29aΔ ± 3	17b ± 2	164aΔ ± 26	94bc ± 23	65bΔ ± 17	31bc ± 10
30 (t_3)	37aΔ ± 6	16b ± 5	231bΔ ± 40	137abcΔ ± 57	45abΔ ± 12	40abΔ ± 14
40 (t_4)	11bΔ ± 2	4c ± 1	29cΔ ± 10	50cΔ ± 17	21cΔ ± 5	13cΔ ± 5
	ferulic acid*		coumaric acid malonyl-hexose **I**		coumaric acid malonyl-hexose **II**	
0 (t_0)	6aΔ ± 2	6aΔ ± 2	15abΔ ± 4	15aΔ ± 4	32abΔ ± 9	32aΔ ± 9
10 (t_1)	6.9aΔ ± 0.5	15b ± 3	10acΔ ± 2	25b ± 9	18cΔ ± 4	37a ± 7
20 (t_2)	3.1bΔ ± 0.8	11bc ± 2	16abΔ ± 2	3c ± 1	38bΔ ± 5	8b ± 4
30 (t_3)	7aΔ ± 2	12bΔ ± 3	23bΔ ± 9	5c ± 2	24acΔ ± 8	11bΔ ± 4
40 (t_4)	1.3bΔ ± 0.2	7ac ± 3	6cΔ ± 2	0.6c ± 0.2	15cΔ ± 5	1.1b ± 0.3
	epicatechin*		**pantothenic acid***		**abscisic acid***	
0 (t_0)	21aΔ ± 9	21aΔ ± 9	14abΔ ± 3	14aΔ ± 3	12abΔ ± 2	12aΔ ± 2
10 (t_1)	2.2bΔ ± 0.8	17a ± 6	13.0abΔ ± 0.4	11aΔ ± 3	16aΔ ± 5	17bΔ ± 2
20 (t_2)	19aΔ ± 4	10a ± 3	11aΔ ± 3	14aΔ ± 3	12abΔ ± 3	16bΔ ± 3
30 (t_3)	32cΔ ± 6	21aΔ ± 10	14abΔ ± 1	17aΔ ± 4	11.9abΔ ± 0.7	15abΔ ± 2
40 (t_4)	7bΔ ± 2	16a ± 5	16bΔ ± 3	15aΔ ± 2	9bΔ ± 1	13ab ± 2

Concentrations are expressed as mean ± SD. n = 10; *Compounds whose identity was confirmed by using pure standards; I and II are isomers of the same compound; Different letters in the same column (for each analyte) shows statistical differences ($p \leq 0.05$) among the diverse time-points; different symbol (Δ) –meaning presence of the symbol in a column and absence of it in the other- at the same line shows statistical differences ($p \leq 0.05$) when comparing on-tree maturation *vs*. cold storage.

introduction. Moreover, some of these compounds have not been previously determined in avocado samples. As reported in the quantitative table, the average concentrations for avocado samples at t_0 (the beginning of the study) were as follows: 36 ± 6 mg Kg^{-1} DW of *p*-coumaric acid, 6 ± 2 mg Kg^{-1} DW of ferulic acid, 21 ± 9 mg Kg-1 DW of epicatechin, 14 ± 3 mg Kg^{-1} DW of pantothenic acid, and 12 ± 2 mg Kg^{-1} DW of abscisic acid. As far as coumaric acid derivatives are concerned, the concentrations found were 165 ± 36 and 54 ± 14 mg Kg^{-1} DW for coumaric acid hexose isomers I and II, respectively, and 15 ± 4 and 32 ± 9 mg Kg^{-1} DW for coumaric acid malonyl-hexose I and II, apiece. In the following sub-sections the results will be analyzed with more detail by grouping the compounds by chemical families, paying attention to possible trends and comparing the effect of the two strategies considered in this research on the final fruit composition (at edible ripeness).

3.3.1. Phenolic acids and related compounds

The hydroxycinnamic acids are abundant in the plant cell walls and are characterized by their high antioxidant capacity (Meyer & Frankel, 2001). They are usually accumulated in higher amounts in avocado pulp with other hydroxybenzoic acids and procyanidins (Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estevez, 2011; Rosero, Cruz, & Osorio, 2019). In the current study, initial concentration levels of *p*-coumaric acid were notably higher than those of ferulic acid (36 ± 6 mg Kg^{-1} DW and 6 ± 2 mg Kg^{-1} DW, respectively). These values were of the same order of magnitude as those previously described in *Hass* avocados and other varieties by different authors (Contreras-Gutiérrez et al., 2013; Hurtado-Fernández et al., 2016; López-Cobo et al., 2016), although, as explained above, a comparison is difficult to make due to the diverse factors that affect the concentration of bioactive substances in this fruit. After 30 days on-tree maturation, levels of *p*-coumaric acid remained relatively stable, with a final significant decrease ($p \leq 0.05$) of about 68% on the last time-point (40 days). On the contrary, for cold storage, a pronounced decline was observed (after an initial slight increase) for the concentrations of this metabolite over time. About 53% reduction in the levels of this phenolic acid was already observed after 20 days, reaching an 88% reduction at the last considered time-point. A comparable observation was reported in a previous study on avocado ripening process with a similar declining behaviour in *p*-coumaric acid (in that case the authors considered just two ripening stages) (López-Cobo et al., 2016). In general, the concentrations of this phenolic acid in the fruit at the ready-to-eat stage were higher when the avocados had remained on the tree for longer (except for the last time-point considered (t_4), which falls within the late harvesting period of *Hass* in Spain.).

Ferulic acid showed a different behaviour than *p*-coumaric acid. The evolution of its concentration in avocados that remained longer in the tree was not clear, although the concentration after 40 days was significantly lower (1.3 ± 0.2 mg Kg^{-1} DW) than the one at the initial time-point. After cold storage, ferulic acid concentrations increased significantly ($p \leq 0.05$) during the first 30 days, reaching a value similar to the initial one at the end of the study (40-days' time-point) with 7 ± 3 mg Kg^{-1} DW.

From the metabolites considered within the current study, the isomers of coumaric acid hexose were those found at higher concentrations in the avocado mesocarp; levels of isomer I varied from 165 ± 36 to 29 ± 10 mg Kg^{-1} DW for the on-tree longer maturation trial and from 165 ± 36 to 50 ± 17 mg Kg^{-1} DW for the cold storage. An initial but non-significant increase was observed in cold storage, whereas the opposite trend was found in the tree. On successive days, the concentration of hexose (isomer I) progressively raised in the tree until the fourth time point (30 days) and then suddenly declined significantly. Coumaric acid hexose isomer II was found at more or less stable concentration levels in the tree for 30 days, and after that, the found amount decreased considerably (21 ± 5 mg Kg^{-1} DW). In contrast, in the cold storage there was a relatively steady decline after the first 10 days for this analyte. There were only statistically significant differences in the concentration of this compound between on-tree *vs*. cold chamber at t_2 (20 days). The protection exerted by the tree on the fruit could have led to a slightly superior stability compared to the fruits stored in a cold chamber.

The isomer I of coumaric acid malonyl-hexose exhibited a very similar behaviour to coumaric acid hexose I (both on-tree and cold storage). The same was evident for coumaric acid malonyl-hexose II when compared to coumaric acid hexose II.

Results from this section might reveal that the phenolic acids and related compounds accumulate during the early and mid *Hass* harvesting season in Spain (27th February to 29th March). However, for fruit harvested later (4th April), the amount of these compounds is lower. On the contrary, a decrease in the concentration of phenolic acids and related substances occurs when avocado fruit is stored under cold conditions for more than 10 days.

3.3.2. Other analytes of interest belonging to different chemical categories

Epicatechin is a flavonoid that belongs to the flavan-3-ol family and it has been related with neuroprotective and antioxidant effects (Bhuyan et al., 2019; Ortega-Arellano et al., 2019). Its initial concentration was 21 ± 9 mg Kg^{-1} DW, similar to the value (15 mg Kg^{-1} DW) reported in a previous study for the same avocado variety (Hurtado-Fernández,

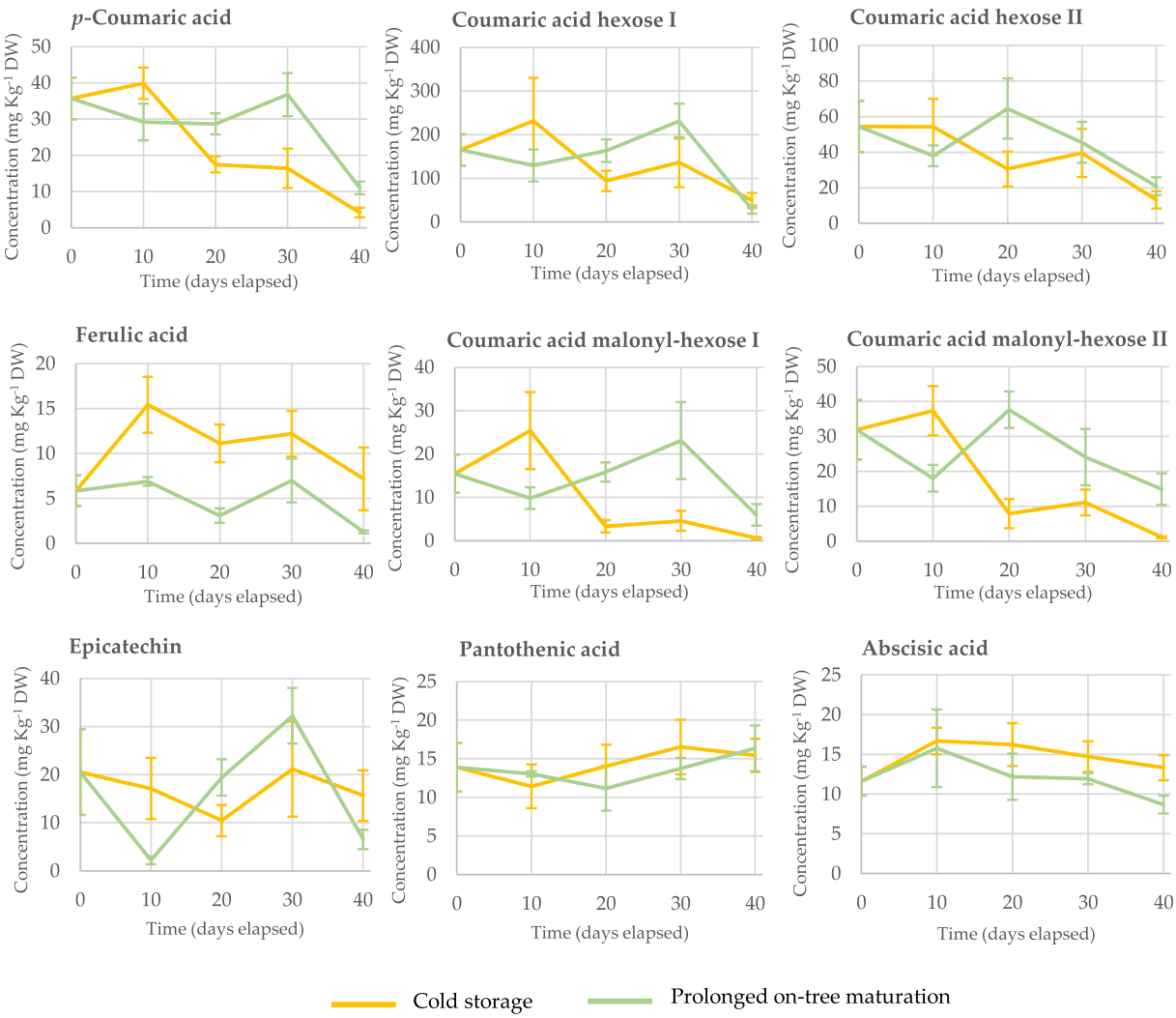

Fig. 1. Effect of the two management strategies on the quantitative evolution of phenolic acids or related substances (*p*-coumaric and ferulic acid, and hexoses of coumaric acid), epicatechin (flavonoid), pantothenic acid (vitamin) and abscisic acid (phytohormone) over a period of 40 days.

Pacchiarotta, Mayboroda, Fernández-Gutiérrez, & Carrasco-Pancorbo, 2014). Throughout the maturation process in the tree or softening in the cold chamber, the quantitative evolution of epicatechin was not clear, so it was not possible to establish a clear pattern. The evolution was similar in both cases, showing upward and downward fluctuations. However, only for fruit from extended tree maturation, some significant differences were observed during the period covered by this research (with a minimum concentration at 10 days, 2.2 ± 0.8 mg Kg^{-1} DW, and a maximum at 30 days after the beginning of the harvest season, 32 ± 6 mg Kg^{-1} DW).

For pantothenic acid (vitamin B_5), the initial quantitative level was lower than the one for epicatechin, being present at a concentration of 14 ± 3 mg Kg^{-1} DW. This vitamin is a precursor of coenzyme A synthesis and it is distributed on animal and vegetal kingdoms. Its consumption is related, for instance, with certain beneficial effects on the skin status (Djerassi, 1993). The quantitative variation of pantothenic acid over the considered period was not notable in any case (neither in the tree nor in cold storage under controlled conditions). The fact just described would allow hypothesizing that this vitamiṅs levels are not conditioned by the pre- and post-harvest management conditions.

Abscisic acid was initially found in amounts of 12 ± 2 mg Kg^{-1} DW. For samples collected after a longer time in the tree, its concentration did not vary significantly over the time span considered in this study. Literature describes that the abscisic acid accumulation on mesocarp depends on the presence of ethylene in the external environment, with the highest concentrations observed just after the peak production of this volatile plant hormone (Meyer, Chope, & Terry, 2017). In addition, it has been reported that abscisic acid is also affected by the external stress and other factors (Bower, Cutting, & Van Lelyveld, 1986). The ethylene produced by the avocado fruit when is detached from the tree could induce a greater stimulation, production and preservation of abscisic acid. The fruits that were stored in cold chamber could, therefore, exhibit significant differences over the ripening due to the enzyme activity and the poorer air circulation. What was observed in the current study for cold stored fruit was an initial increase of abscisic acid concentration and a reversion to starting levels after 30 days. Higher concentrations of this compound were determined in the fruit stored in the cold ripening chambers (t_4) when compared with the avocados which were attached to the tree for a longer period.

Chirinos et al. (2021) have recently carried out a study in which they evaluated *Hass* avocado fruits from two harvests which were subjected to hydrothermal treatment or left untreated and then stored for 30 and 50 days in a controlled atmosphere (with subsequent ripening at ~ 20 °*C*). Found amounts of abscisic acid at edible ripeness and some of the trends described for the concentration of this analyte are in good agreement with the results included in this contribution.

In addition, Hurtado-Fernández et al. (2016) conducted a study were *Hass* avocado metabolic composition was evaluated over the harvest

season; in the samples analyzed by those authors, the initial concentration values were somewhat higher than those described here for epicatechin, but lower for pantothenic acid and abscisic acid. This is perfectly reasonable considering the variability of avocado's composition between seasons and harvest periods.

3.4. Total content of metabolites in the evaluated samples

Total metabolite contents were calculated as the sum of the nine metabolites quantified at each time-point and for both strategies. Standard deviations were established as the square root of the quadratic sum of the corresponding standard deviation for each individual compound and expressed in mg Kg^{-1} DW. Summative calculations were also made for phenolic acids. Fig. 2 shows the corresponding representations using bar diagrams; on the left-hand side (Fig. 2a) the overall sums have been represented (at the different time-points for the two management strategies) and on the right-hand side (Fig. 2b) the sum of the phenolic acids can be seen. As phenolic acids are, in any case, the most abundant group of metabolites considered in this work, both representations are very similar. Statistically significant variations were observed according to the different management strategies, monitoring the maximum total metabolites content after 10 days of storage in a cold chamber and after 30 days of prolonged on-tree maturation. Altogether, there was a constant increase in total metabolites content while the fruit were attached to the tree (t_0, t_1, t_2 and t_3). In contrast, in cold-stored avocados, there was an initial increase in overall content (at 10 days) but a continued decrease thereafter. In both cases, a significant and pronounced final decline was observed (at the 40-days' time-point).

In the introductory section, very interesting articles that focused either on the determination of different parameters/compounds over the harvesting season or during the softening of the fruit were cited. All these works made very relevant contributions (Arpaia et al., 2015; Campos et al., 2020; Meyer et al., 2017; Obenland et al., 2012; Ozdemir & Topuz, 2004; Villa-Rodríguez, Molina-Corral, Ayala-Zavala, Olivas, & González-Aguilar, 2011; Wang et al., 2012), however, almost all of them addressed the determination of other compounds (fatty acids, sugars, volatile compounds…) or the measurement of other parameters (sensory attributes, antioxidant activity, dry matter, days to ripen, total phenolic compounds, etc.). To the best of our knowledge, no work has been published with this experimental design evaluating the quantitative evolution of the nine metabolites selected in this research (certainly related to nutritional quality of avocado). Thus, the amount of these bioactive compounds that, depending on the pre- and post-harvest management of avocado, reach the consumer has not been described so far.

Therefore, if the aim is to maximize the nutritional quality of the fruit, the optimum harvesting time for *Hass* avocados in Southern Spain (in the temporal interval evaluated here and under the conditions contemplated in this research) would be at the end of March. For continental exports, the optimum cold storage time for avocados picked at the end of February would be 10 days, after which the concentration of bioactive substances would decrease. All of the above means that the fruit could be harvested early in order to obtain economic benefits according to the needs of the market, while maintaining a high nutritional value. Moreover, early harvested fruit could remain in storage for up to 30 days without an unacceptable loss of metabolites of interest, although after 10 days the level of bioactives would be lower (for cold storage).

4. Conclusions

The processes that take place in the avocado fruit during maturation and softening has been the subject of much research interest, but an experimental design such as the one envisaged here has not been described before. The perspective of this study was distinctive, as it focuses on finding out the effect of two different management strategies on the final composition of the avocado fruit that reach the consumer and, thus, to some extent, on their potential health benefits. The effect of both harvest date and post-harvest management (cold storage) on the metabolic profile of *Hass* avocados grown in Spain between February and April (considering an interval of 40 days) was evaluated, taking into account nine metabolites of bioactive interest (and their summation). The two scenarios explored in this paper would be possible as long as domestic consumption or relatively short-distance exports are involved.

Our results indicate that the concentration of the most abundant metabolites of those evaluated (phenolic acids and related substances) rises over the early and mid *Hass* harvesting season in Spain (27th February–29th March). It is also possible to state that a drop in the concentration of phenolic acids and related substances occurs when avocado fruit is stored under cold conditions for more than 10 days, although it should be noted that up to about 30 days the fruit could be stored without a very significant decline in terms of bioactive substances. Pantothenic acid did not show drastic changes in any case, and for epicatechin it was not possible to establish a clear pattern. In the case of abscisic acid, higher concentrations were determined in avocados stored in the cold ripening chambers, probably due to the accumulation of ethylene in the environment that stimulates its synthesis. In an overall view, considering the evolution observed during the first 30 days of the study, the total content of bioactive compounds increases for avocados that remain longer on the tree, while it decreases for those avocados ripened in the chamber.

We firmly believe that this type of research is necessary, firstly, to delve further into "knowing what we eat" and secondly, to better understand the physiology and ripening phenomena of this interesting tropical fruit.

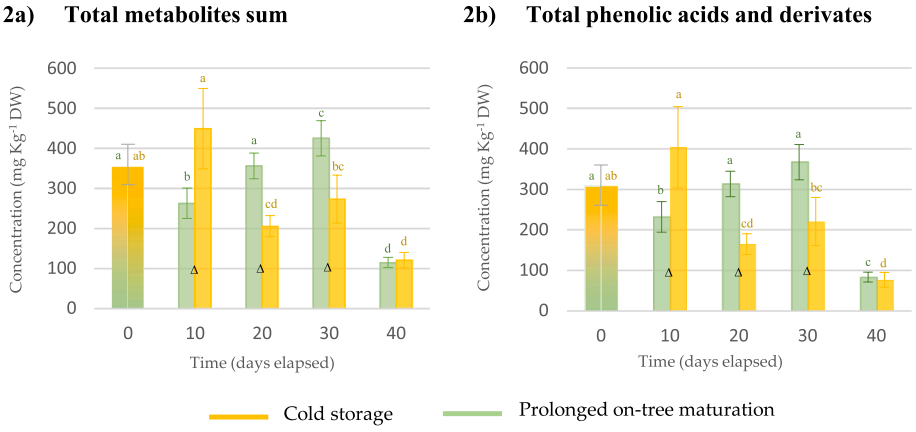

Fig. 2. Bar diagram representing the sum (mg Kg^{-1} DW) of all the avocado metabolites evaluated within this study (2a) and phenolic acids and derivatives (2b) for on-tree extended maturation and storage in a refrigerated chamber, at ten days intervals. Different letters on the same colour above the bars of the chart indicate statistically significant differences ($p \leq 0.05$) after applying one-way ANOVA followed by Turkey's test to compare the concentration values among the different time-points belonging to the same strategy (on-tree or cold storage). The presence of the symbol (Δ) reveals statistically significant differences ($p \leq 0.05$) when applying ANOVA followed by Turkey's test comparing the results of the two management strategies at the same time-point.

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CRediT authorship contribution statement

Irene Serrano-García: Conceptualization, Methodology, Formal analysis, Data curation, Investigation, Writing – original draft, Writing – review & editing. **Elena Hurtado-Fernández:** Conceptualization, Writing – review & editing. **José Jorge Gonzalez-Fernandez:** Resources, Writing – review & editing. **José Ignacio Hormaza:** Resources, Writing – review & editing. **Romina Pedreschi:** Resources, Writing – review & editing. **Patricia Reboredo-Rodríguez:** Methodology, Formal analysis, Data curation, Writing – review & editing. **María Figueiredo-González:** Methodology, Formal analysis, Data curation, Writing – review & editing. **Lucía Olmo-García:** Conceptualization, Methodology, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Alegría Carrasco-Pancorbo:** Conceptualization, Resources, Methodology, Investigation, Supervision, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133447.

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