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New insights into the heterogeneous ripening in Hass avocado via LC–MS/MS proteomics

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ABSTRACT

Hass avocado postharvest ripening heterogeneity creates several logistics problems at destination markets. Although several approaches mainly based on targeted analysis have been undertaken to broaden our understanding of the potential causes of this postharvest ripening heterogeneity, still the secret is not yet unveiled. In this study biopsies from individual fruit displaying contrasting ripening behavior (slow vs fast) and subjected to different storage conditions (immediately after harvest, 30 d regular air at 5 °C and 30 d at 5 °C and 4 kPa O_2 and 6 kPa CO_2) were analyzed for targeted fatty acid analysis and cell wall enzyme activity combined with a more integrative approach based on gel free proteomics LC–MS/MS. The high throughput proteomics was capable of discriminating between slow and fast ripening avocado fruit. Proteins that were mainly less abundant in the slow ripening phenotype were annotated to correct protein folding, translation and de novo synthesis and higher abundant proteins were annotated to amino sugar and nucleotide metabolism, detoxification and stress response and proteolysis. Our study opens new insights into the causes of heterogeneous ripening and may provide new markers for avocado ripening at harvest and after prolonged storage.

1. Introduction

Hass is the main avocado cultivar commercialized worldwide and its international trade has doubled in the span of 6 years (Fruit Trop, 2015). Mexico remains the major producer and exporter in the world, followed by Peru and Chile. Hass avocados in Europe and USA are usually sold as´triggereá (ripened to a certain degree) and´ready to eaí. These formats demand homogenous and high consistency of quality in each batch. However, avocado physiology is complex and several pre- and postharvest factors lead to postharvest ripening heterogeneity (Pedreschi et al., 2014; Hernández et al., 2016). The most characteristic feature of this situation is the difference in days to reach edible ripeness within a box at the retail and consumer levels (Pedreschi et al., 2014).

The flowering period can last up to three months resulting in a wide variability in age among fruit within a tree at harvest (Lewis, 1978). Avocados do not ripen on the tree and can remain for more than 12 months, which is far beyond the time needed to reach physiological maturity and ability to ripen (Woolf et al., 2004). The inability to ripen on the tree has been linked to the flow of inhibitory compounds C_7 sugars (mannoheptulose and perseitol) from the leaves to the fruit controlling and/or triggering the ripening process (Liu et al., 1999, 2002; Bertling and Bower, 2005; Landahl et al., 2009). In addition, C_7 sugars are energy sources for respiration during postharvest life (Tesfay et al., 2012). Thus, the amount of these C_7 sugars (mannoheptulose) at harvest was postulated as an indicator of the ripening speed of the fruit (Blakey et al., 2012). However, in a study of middle season fruit no correlation was found between the amount of C_7 sugars at harvest and the speed of ripening (Pedreschi et al., 2014). In this 2014 study, metabolites associated to fatty acid, amino acid and cell wall metabolism differed between very fast and very slow ripening fruit. These previous reported results on middle season fruit opened room to further analyze and validate the role of fatty acids and cell wall disassembly enzymes involvement in the observed postharvest Hass avocado ripening heterogeneity.

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Fatty acids, especially of the monounsaturated type, are largely present in avocado mesocarp tissue. Major changes in fatty acid profiles in Hass avocado have been reported to occur during growth and development, with a marked increase in oleic acid as the fruit matures. Only small changes in oil composition have been reported during postharvest ripening, and thus fatty acids are not considered to be a major substrate for respiration (Ozdemir and Topuz, 2004; Meyer and Terry, 2006) and their role in ripening heterogeneity is not clear up to date.

Integrative approaches to understand ripening heterogeneity are very limited (Pedreschi et al., 2014). In many non-model plants and crops, till now gel based proteomics was powerful but low throughput approach (Carpentier et al., 2008; Agrawal et al., 2013). Genomic resources for avocado are not massive, but recently, the transcriptome of *Persea americana* var. drymifolia for different organs and ripening stages has been released with a total of 67 000 unigenes and 34 128 proteins (Ibarra-Laclette et al., 2015). Also the transcriptome of the mesocarp tissue of Hass avocado at different developmental and ripening stages has been published (Kilaru et al., 2015). Thus, performing a gel-free proteomics approach to study avocado ripening contrasting phenotypes becomes feasible (Carpentier and America, 2014).

In this research, postharvest ripening heterogeneity of Hass avocado was studied with targeted metabolite and enzyme analysis and untargeted gel-free proteomics analyzing contrasting phenotypes under different commercial storage conditions. The objectives of this work were: (i) to describe the biochemical and physiological implication of fatty acid metabolites and key cell wall disassembly enzymes in an attempt to identify early stage quality markers of ripening heterogeneity and (ii) to assess differences at the protein level of samples with contrasting ripening phenotype under different commercial storage conditions.

2. Materials and methods

2.1. Plant material and treatments

Three hundred Hass avocado fruit per season defined based on dry matter content (DM) (early < 23–26% DM; middle > 26%–30% DM and late > 30% DM) were harvested from 10 previously marked trees from La Palma Experimental Station located in Quillota, Chile. Trees were grown under the same environmental and crop management standard commercial conditions. The fruit were immediately transported to the laboratory facilities and cooled to 7 °C overnight. For each season, fruit were divided in three postharvest treatments composed of 100 individual fruits each: (i) recently harvested (H), (ii) 30 d storage at 5 °C in regular air (RA) and 30 d storage at 5 °C and 4 kPa O_2 and 6 kPa CO_2 controlled atmosphere conditions (CA). Four fruit biopsies of 5 mm diameter from the equatorial part of the fruit without skin were taken from each individual fruit after exposure to the three postharvest treatments, biopsies were sealed with a combination of wax and vaseline as previously described by Pedreschi et al. (2014). Then, after the postharvest storage treatments, fruit were ripened at 20 °C and 60–70% relative humidity and ripening was followed individually in each fruit until the 'ready to eat' (RTE) stage was attained (firmness value of 4.44–13.3 N). Based on the ripening behavior of each individual fruit in each independent postharvest treatment and season as performed by Pedreschi et al. (2014), fruit were classified and the extreme contrasting ripening behavior fruit (4 independent replicates): 'slow ripening' vs 'fast ripening' fruit were subjected to further targeted fatty acid and cell wall disassembly enzymatic analysis. Only early season fruit ('slow' vs 'fast ripening' fruit) were subjected to gel free proteomics analysis (Table 1).

2.2. Ripening heterogeneity assessment

The variation in time to reach edible ripeness (RTE) for the different season fruit (early, middle and late) and postharvest treatment (H, RA and CA) were evaluated through multiple comparison of squared residuals using non-parametric method of Brown and Forsythe (1974). The squared residuals were calculated using the median of each treatment since it is more suitable for non-symmetric distribution (or heavy tailed distributions) and large samples than using the mean of each treatment (Banga and Fox, 2013). Then, graphical multiple comparisons of squared residuals based on Fisher's least significant difference were calculated using one-way ANOVA (95% confidence) as described by Fuentealba et al. (2016). Squared residuals and multiple comparisons were performed in Excel 2013 (Microsoft Inc.) and Statgraphics Centurion XVII software (StatPoint Technologies Inc., Rockville, Maryland, USA), respectively. In addition, data distribution histograms of each postharvest treatment were performed using Minitab 17.1.0 (Minitab Inc.).

Table 1
Average days to reach edible ripeness corresponding to a (firmness value of 4.44–13.3 N) at 20 °C and 70% RH (RTE) of 'fast' or 'slow' Hass avocado fruit. Fruit were taken from early, middle and late harvest seasons and examined after harvest or after 30 d storage in and regular air or controlled atmosphere storage conditions.

Harvest Season	Storage condition	RTE (d)	
		Fast	Slow
Early (23–26% DM)*	Harvest*	13	36
	RA	4	6
	CA*	5	19
Middle (> 26–30% DM)	Harvest	12	19
	RA	3	17
	CA	5	18
Late (> 30% DM)	Harvest	7	28
	RA	1	14
	CA	3	14

* For gel free proteomics, only early season fruit was analysed. RA: regular air storage for 30 d at 5 °C and CA: controlled atmosphere storage at 5 °C and 4 kPa O_2 and 6 kPa CO_2 for 30 d.

2.3. Physiological parameters

2.3.1. Fruit quality parameters

Flesh firmness was measured at the equatorial part without skin on opposite sides using a penetrometer equipped with an 8 mm plunger tip. Dry matter content was determined at the ready to eat stage for each individual fruit by drying the samples at 103 °C for 24 h and correcting for water loss during storage.

2.3.2. Respiration rate and ethylene production rate

A total of 150 randomly picked fruit per season (early, middle and late) corresponding to 50 fruit per postharvest treatments: harvest (H), regular air (RA) and controlled atmosphere (CA) conditions were taken for respiration rate and ethylene analysis before biopsies were taken. For ethylene and respiration, each independent fruit was placed in 1.6 L plastic containers and sealed for 3 h at 20 °C. Then, 1 mL of gas was taken from the headspace and injected into a gas chromatograph (Shimadzu GC 8A, Tokyo, Japan) equipped with an alumina column (Supelco 80/100 Porapak of 75 cm × 5 mm × 3 mm dimensions) and flame ionization detector (FID). The oven and injector temperatures were 40 °C and 150 °C, respectively. Results were expressed as μg $C_2H_4\ kg^{-1}\ h^{-1}$. Respiration rate at 20 °C was measured by injecting 1 mL of gas from the headspace to a gas analyzer (PBI-Dansensor Checkmate 9900, Ringsted, Denmark) and expressed as mg $CO_2\ kg^{-1}\ h^{-1}$.

2.4. Targeted fatty acid identification and quantification

Powder freeze dried (200 mg) mesocarp avocado tissue from the