Postharvest Biology and Technology 92 (2014) 172–179

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Metabolomics analysis of postharvest ripening heterogeneity of 'Hass' avocadoes

Romina Pedreschi[a,f,*], Pablo Muñoz[b], Paula Robledo[b], Cecilia Becerra[b], Bruno G. Defilippi[b], Henriëtte van Eekelen[c,d,e], Roland Mumm[c,d,e], Eelke Westra[a], Ric C.H. de Vos[c,d,e]

[a] Food & Biobased Research, Wageningen University and Research Centre, The Netherlands
[b] Unidad de Postcosecha, Instituto de Investigaciones Agropecuarias (INIA-La Platina), Santiago, Chile
[c] Plant Research International, Wageningen University and Research Centre, The Netherlands
[d] Centre for Biosystems Genomics, P.O. Box 98, 6700 AB Wageningen, The Netherlands
[e] Netherlands Metabolomics Centre, Einsteinweg 55, 2333 CC Leiden, The Netherlands
[f] Pontificia Universidad Católica de Valparaíso, Escuela de Agronomía, Chile

ARTICLE INFO

Article history:
Received 30 October 2013
Accepted 25 January 2014

Keywords:
Persea americana
Metabolomics
LC–MS
GC–MS
Fatty acids
Isoprenoids

ABSTRACT

The complex physiology of 'Hass' avocado renders its postharvest ripening heterogeneous and unpredictable. Several approaches have previously been undertaken to broaden our understanding of the causes of this postharvest ripening heterogeneity but without much success. In this study, a fruit biopsy methodology was undertaken to sample mesocarp tissue from a series of individual avocado fruit while following individual fruit postharvest ripening characteristics without significantly disturbing their metabolism. Using both targeted and untargeted metabolomics approaches, we analyzed the metabolite profiles of the biopsies in order to get more insight into the biochemical mechanisms underlying 'Hass' avocado ripening heterogeneity. While C_7 sugars (mannoheptulose and perseitol), dry matter and total Ca^{2+} were not correlated with time to reach edible ripeness, untargeted metabolomics profiling of polar and semi-polar compounds (based on GC–MS and LC–MS platforms), revealed several metabolites, mainly amino acids, that were related to ripening heterogeneity. In addition, analysis of fatty acids revealed linoleic acid to be differentially accumulating. In general, slowest ripening avocados had lower amounts of precursors of metabolites involved in key metabolic pathways. Our study indicates that comprehensive metabolomics may provide new markers for avocado ripening stage at harvest, and may give more insight into the complex ripening physiology of this fruit.

1. Introduction

The complex physiology of 'Hass' avocado can be seen in the heterogeneity observed in postharvest ripening (referred as 'days to reach edible ripeness'), which creates severe logistical problems for marketers and inconsistent quality delivery to consumers. Probably the most characteristic feature of this situation is the differential colour observed in a commercial box, known as "checkerboard" after long and cold transport (Rose, 2003), and later in the days to reach edible ripeness. The avocado flowering period can last up to three months, causing a wide variability in age among fruit at harvest time (Lewis, 1978). Avocados can hang on a tree for more than 12 months, which is far beyond the time needed to reach physiological maturity or ability to ripen (Woolf et al., 2004). Furthermore, avocado ripening only happens several days after the fruit has been detached from the tree, showing a very high respiration rate at room temperature. Thus, to prolong storability and avoid chilling injury, 'Hass' avocados are kept at temperatures not above 5 °C in air or controlled atmosphere conditions.

A flow of inhibitory compounds from the leaves to the fruit has been postulated to be involved in this ripening inhibition on the tree. It has previously been postulated that C_7 sugars (mannoheptulose and perseitol) play a major role in controlling/triggering the ripening process (Liu et al., 1999, 2002; Bertling and Bower, 2005; Landahl et al., 2009), and that their reduction to a certain threshold would be a physiological prerequisite for fruit ripening. However, the potential involvement of these C_7 sugars in triggering/inhibiting ripening when the fruit is detached from the tree is

* Corresponding author at: Escuela de Agronomía, Pontificia Universidad Católica de Valparaíso, Chile.
E-mail addresses: romina.pedreschi@ucv.cl, r_pedreschi@yahoo.com (R. Pedreschi).

0925-5214/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.postharvbio.2014.01.024

still unexplained. The inherent huge biological variation of 'Hass' avocados already on a single tree (due to different age of the fruit and other preharvest factors) demands thoughtful experiments to properly sample fruit for specific research questions.

Fruit biopsy has previously been used as a methodology to sample small amounts of mesocarp tissue without disturbing normal avocado metabolism (Kanellis et al., 1989; Gamble et al., 2010; Obenland et al., 2012; Blakey et al., 2012). Fruit biopsy combined with both non-destructive measurements to follow postharvest ripening and high throughput approaches at different cellular control levels (e.g., transcriptomics, proteomics, metabolomics) may be the next step to get a better understanding of the physiology behind the heterogeneity observed in avocado ripening.

Metabolomics is increasingly used in plant research to provide detailed information on the similarities and differences in metabolite composition between samples. It also integrates post-transcriptional and translational information which directly determines the phenotype. Many studies have focused on preharvest fruit physiology (e.g., growing conditions, developmental stages, characterization of different cultivars, etc.) and postharvest biotic and abiotic stresses (e.g., temperature, gas conditions) in order to gain more insight into the biochemical mechanisms involved (Carrari et al., 2006; Pereira et al., 2006; Guy et al., 2008; Jahangir et al., 2008; Pedreschi et al., 2009; Rudell et al., 2009; Ballester et al., 2013; Luengwilai et al., 2012; Bernillon et al., 2013). Several platforms are available for the large-scale analysis of metabolites, such as GC or LC coupled to mass spectrometry or nuclear magnetic resonance spectroscopy (NMR). Given the nature of the different metabolites, a combination of different platforms will give the broadest insight into the plant metabolome (Cevallos-Cevallos et al., 2009; Hall, 2011).

Dry matter content, the gold standard avocado harvest index, which correlates very well with oil content, is extensively used to harvest 'Hass' avocados to comply with the minimum standards to guarantee good eating quality. Non-destructive tools such as near infrared spectroscopy are being implemented on-line in packing houses in an attempt to better segregate 'Hass' avocados at the gate and reduce the observed postharvest ripening heterogeneity in long distance markets. In the present study, a comprehensive metabolomics approach combined with classical postharvest analytical approaches was used to get more insight into the mechanisms involved in the ripening heterogeneity observed within 'Hass' avocados. The main objectives of this study were: (i) to establish a proper fruit biopsy sampling approach for 'Hass' avocado, (ii) to relate the levels of specific metabolites, determined through a combination of targeted and untargeted metabolomics approaches in individual fruit with their ripening profiles in order to explain the ripening heterogeneity in 'Hass' avocados. The results of this study not only provide more information on the biochemical heterogeneity of 'Hass' avocados but also identify those metabolites that are potentially involved in the heterogeneity, which may help to implement non-destructive tools or postharvest protocols to reduce heterogeneity in practice.

2. Materials and methods

2.1. Fruit material and chemicals

One hundred avocados (*Persea americana* Mill. cv Hass) were sampled from the same tree from a commercial orchard located at Llay-Llay, Región Valparaiso, Chile. The fruit were immediately transported to the laboratory facilities at Instituto de Investigaciones Agropecuarias (INIA) and cooled down to 5 °C overnight. The most suitable fruit biopsy approach for 'Hass' avocados was tested in The Netherlands with fruit from an importer and later on, the results were confirmed with local fruit in Chile.

D-(−) fructose, D-mannoheptulose and perseitol were obtained from Sigma–Aldrich (St. Louis, USA), while D-(+) glucose and sucrose were obtained from Supelco Analytical (Bellefonte, USA).

2.2. Biopsy methodology and experimental set-up

Fruit biopsies of different diameter sizes (5, 6.5 and 7.5 mm) and biopsy numbers (1, 2 and 4) were taken from the equatorial part of the fruit with a core borer. Thirty individual avocados were used per treatment. Treatments corresponded to a control (no biopsies taken) and biopsy treatments (combinations of number (1, 2 and 4) and size (5, 6.5 and 7.5 mm) on each individual fruit. Ripening (measured as loss of firmness, 0–5 hedonic scale (0 = extremely hard, 1 = first softening, 2 = little soft; 3 = soft-ready to eat, RTE (4.44–13.3 N), 4 = very soft and 5 = extremely soft)) was followed on each independent fruit at 20 °C. The experiment was repeated 3 times. Once the fruit biopsy approach was set-up (4 mm × 5 mm samples), it was used to sample mesocarp avocado tissue from 100 individual fruit immediately after harvest in Chile. The tissue sample (mesocarp tissue without peel) from each individual avocado was snap frozen in liquid nitrogen and stored at −80 °C. A part of the tissue was freeze-dried and the rest kept at −80 °C for further analysis. After taking the biopsies, the fruit were sealed with a combination of inert wax and vaseline without significantly disturbing fruit ripening. Targeted metabolomics analysis was carried out with all the biopsy samples (C_7 sugars, total Ca^{2+} and dry matter were measured on each individual fruit). C_7 sugars were measured on the biopsy tissue taken at the start and calcium and dry matter were measured at the ready to eat stage or edible ripeness (hand firmness value of 3 or 4.44–13.3 N) and corrected for water loss during storage.

Based on the ripening behaviour of each individual fruit, 5 different patterns were identified based on cluster analysis. For each pattern or cluster identified, 3 individual fruit were used to analyze polar and non-polar metabolites. The complete experimental design is shown in Fig. 1.

2.3. Analysis of C_7 and C_6 sugars, total Ca^{2+} and dry matter

C_6 and C_7 sugars were determined following the protocol of Pérez et al. (1997) with some modifications. Sugars were extracted from ~1 g (FW) frozen and previously powdered avocado mesocarp tissue. The sample was mixed with 5 mL of cold 95% ethanol and let to stand at room temperature for 3–5 min. The homogenate was centrifuged at 15,180 × *g* for 20 min and vacuum-filtered through two layers of Whatman 1 paper. The residue was further washed with 80% ethanol and the volume adjusted to 10 mL which was dried under a nitrogen stream at 50 °C. The dried residue was dissolved in 2 mL of H_2SO_4 with 0.05% EDTA and then loaded on an activated Sep-Pak cartridge. The sugars were subsequently eluted with 1 mL of H_2SO_4 with 0.05% EDTA and filtered through a 0.45 μm filter prior to HPLC analysis. HPLC was carried out on a Kromasil 100 $5NH_2$ column of 250 mm × 4.6 mm (Akzo Nobel, Bohus, Sweden) using as mobile phase 77% acetonitrile and 23% HPLC grade water previously degassed and ultrasonicated. The flow rate was kept at 1.8 mL min^{-1} under a pressure of 13.2 kPa. A total of 20 μL of sample were injected. Sugars were detected by an evaporative light scattering detection (ELSD) detector (Sedex 60 lt ELSD) with an interface LCNET II/ADC (Jasco, Japan). C_6 and C_7 sugars were quantified based on calibration curves built with authentic standards.

Dry matter was carried out by drying the sample in an oven at 90 °C × 24 h taking a representative sample of mesocarp avocado tissue as described by Meyer and Terry (2010). Samples for total calcium analysis were analyzed by atomic absorption spectrometry according to Kalra (1998) and Temminghoff and Houba (2004). Both dry matter and total calcium were measured at time to reach edible