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Effects of heat shock and nitrogen shock pre-treatments on ripening heterogeneity of Hass avocados stored in controlled atmosphere

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ABSTRACT

Hass avocado ripening heterogeneity generates logistics problems to importers and ripeners due to higher labour costs, inconsistent quality delivery and postharvest losses. The main aims of this research were: (i) to evaluate two postharvest pre-treatments (nitrogen shock N_2 and heat shock) prior controlled atmosphere (CA) on reduction of ripening heterogeneity of Hass avocado without being detrimental to fatty acid profile and (ii) to study the potential metabolic processes implicated in such ripening synchronization with focus on cell wall remodelling and ethylene biosynthetic pathways. Results showed that heat shock prior to CA storage significantly reduced ripening heterogeneity in early and middle season fruit while N_2 + CA did not. Pectin methyl esterase (PME) and polygalacturonase (PG) activity did not display significant differences among treatments. Additionally, none of the treatments altered the fatty acid profile. ACS transcript for early and middle season fruit kept constant during storage for heat + CA, CA and N_2 + CA. ACO instead displayed less abundance after 21 d storage for all treatments of early season fruit. These results point to ripening synchronization in Hass avocado subjected to heat to be related to induction of metabolic processes related to ethylene (biosynthesis), possibly at the action level (receptors) but the efficiency of the heat treatment was related to the maturity stage of the batch.

1. Introduction

Hass avocado consumption increases every year due to its organoleptic, nutritional and functional attributes (Meyer and Terry, 2010). Historically, Mexico remains the main world producer and exporter, exporting during the season 2015/2016 590,000 t to different destinations. During the same season, South American countries such as Peru and Chile exported 190, 000 t and 100,000 t, respectively. Main destinations for Chilean exports are Europe (33%) and USA (9.5%) of the total export volume (Comité de Paltas Hass Chile, 2015).

Most of Hass avocado fruit in Europe and USA are commercialized as “ready to eat” and “triggered” fruit. “Ready to eat” fruit reaches prices 30% higher than fruit sold in bulk at a green stage (Hernández et al., 2016). Consumers are more demanding of a high and consistent quality product (Hofman et al., 2013). Thus, for both niche markets having homogenous batches is important. Hass avocado ripening heterogeneity represents a severe logistic problem in the avocado supply chain for importing countries. This is translated into higher labour costs due to re-sorting of fruit and inconsistency in the delivery of high and consistent quality fruit to consumers (Blakey et al., 2009). Thus, pre-sorting batches based on homogenous ripening would represent a huge improvement to the avocado supply chain.

Hass ripening heterogeneity is associated to an intrinsic condition linked to its complex physiology. It presents a long flowering period, complex hormonal system and its inability to ripen while attached to the tree gives no possibility to base harvest on firmness and colour changes (Bower and Cutting, 1988). Harvest standards for Hass avocado in Chile are based on a minimum oil content of 9% (~23% dry matter content). Previous studies have associated ripening heterogeneity with its inability to ripen on the tree due to mannoheptulose and perseitol naturally present in avocado that translocate from the fruit to the leaves, thus controlling/triggering the ripening process

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(Blakey et al., 2012). However, other relatively recent studies (Pedreschi et al., 2014) could not demonstrate a correlation between the concentration of these C_7 sugars in the mesocarp with the days to ripen or days to reach edible ripeness.

Exporting countries from the southern hemisphere use post-harvest technologies such as refrigeration in normal air conditions or combined with controlled atmosphere (CA) conditions to guarantee fruit quality after the long travel times to their destinations. Postharvest treatments such as heat shock (HS) and water infusion through the pedicel could partially reduce the ripening heterogeneity of Hass avocado batches (Blakey and Bower, 2007; Blakey et al., 2009) but the basis of these treatments on reducing ripening heterogeneity are not fully understood.

Commercially used postharvest technologies can help to synchronize ripening during storage of Hass avocados. Modification of the atmosphere targeting a reduction of the oxygen level through a nitrogen shock retards the ripening process. However, low concentration of oxygen can result in physiological disorders such as abnormal ripening, off-flavours, pulp browning and increase in the contents of ethanol and acetaldehyde (Lara et al., 2010). Retrieved peaches from anoxic N_2 atmospheres ripened normally without any consequence on firmness, flavour and colour (Lara et al., 2010). Maintenance of firmness was due to inhibition of ethylene production. N_2 atmosphere reduces the production of ethylene and it is correlated with low levels of ACO1 probably because N_2 acts similarly as CO_2 as a competitor. In addition, the content of acetaldehyde is increased, and this also inhibits the activity of ACO resulting in a low polygalacturonase activity, thus retarding softening (Lara et al., 2010).

Thermal treatments are required as phytosanitary treatments by some importing countries. Thermal treatments trigger the biosynthesis of heat shock proteins (HSP) in the fruit tissue protecting cellular components (Lurie and Pedreschi, 2014). Water heat treatments (38 °C for 60 min and other combinations of temperature-time) can trigger the biosynthesis of anthocyanins as a protection mechanism, thus causing stains on the skin of the fruit. However, these stains become masked during ripening of the fruit (Blakey and Brower, 2007). Thus, heat treatments are not recommended for green peel avocados such as Fuerte and Pinkerton but very effective for black peel avocados such as Hass (Blakey and Bower, 2007). It has been previously reported that heat treatments on Hass avocado help to reduce the ripening heterogeneity, however, the scientific explanation is missing. These previously mentioned authors hypothesize that this ripening synchronization due to heat treatments can be attributed to a higher synthesis of enzymes, induction of abscisic acid and protection of the ethylene receptors by HSP (Blakey and Brower, 2007).

Up to date, there are huge gaps related to the understanding of the ripening heterogeneity of Hass avocados. Thus, in this research, we address the following objectives: (i) to evaluate the effect of three postharvest treatments (nitrogen shock plus controlled atmosphere, heat shock plus controlled atmosphere and controlled atmosphere) on the reduction of the ripening heterogeneity of Hass avocados and (ii) to study the potentially associated metabolic processes using targeted approaches based on cell wall remodelling enzymes, ethylene biosynthesis transcripts and fatty acids.

2. Materials and methods

2.1. Fruit material and sampling

Fruit were harvested from 10 homogenous avocado trees cv. Hass located in the Experimental Station La Palma, Quillota, Chile. Trees were grown under standard commercial environmental and crop management conditions. The fruit were immediately transported to the laboratory facilities and cooled down to 5° C overnight. Two harvests were performed corresponding to early season fruit (23–26% dry matter, in September 2015) and middle season fruit (> 26–30% dry matter in December 2015). The total number of harvested avocados per season was 300 with an average weight of 170–220 g. Harvest season (early or middle) on the orchard sector was carried out during the commercial harvest. The time for these harvests was based on sampling a determined number of fruits (20) every week during the Hass avocado season.

2.2. Imposed treatments

The imposed postharvest abiotic stresses or treatments corresponded to: (i) heat treatment (HT): 100 fruit per harvest season (early and middle) were immersed in water at 38 °C for 1 h followed by 30 d storage at 5 °C with controlled atmosphere conditions of 4 kPa O_2 and 6 kPa de CO_2; (ii), (2) nitrogen shock (N_2): 100 fruit were held in a closed container injected with nitrogen gas for 24 h reaching concentrations of 1 kPa O_2 and 1 kPa CO_2, and then were stored for 30 d at 5 °C with controlled atmosphere conditions of 4 kPa O_2 and 6 kPa CO_2 and (iii) controlled atmosphere conditions (CA): 100 fruit were stored for 30 d at 5 °C in controlled atmosphere conditions of 4 kPa O_2 and 6 kPa CO_2. During storage, CA conditions were disturbed at days 1, 8, 15 and 21 to sample one biopsy from 50 individual fruits per treatment. Biopsies were sealed with vaseline and wax as previously described by Pedreschi et al. (2014).

After the 30 d storage, avocados cv. Hass were exposed to shelf conditions (20 °C and 60–70% RH) to allow ripening. RTE (time to reach edible ripeness, hand soft equivalent to firmness value of 3.5–8 N) was recorded for each individual fruit. Dry matter was measured on each individual fruit at the RTE stage correcting for water loss.

2.3. Assessment of ripening heterogeneity

To analyse differences in ripening heterogeneity of the different treatments, the method of Brown and Forsythe (1974) and Minitab 17 Statistical Software (State College, PA, USA) with a p value of 0.05 were used. Then, graphical multiple comparisons of squared residuals based on Fisher's least significant difference were calculated using one-way ANOVA (95% confidence) as implemented by Fuentealba et al. (2016). Based on this treatment assessment of heterogeneity, four independent fruit or biological replicates belonging to the homogenous ripening group were chosen for analysis from different sampling times.

2.4. Respiration rate and ethylene production

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 μL 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}. Fifty fruits without biopsy were randomly picked at 1, 8, 15 and 21 d storage for respiration rate and ethylene measurements.

2.5. Fatty acid methyl esters (FAME) analysis

Fatty acid analysis was performed on dried samples from the dry matter determinations for the three treatments. Oil was extracted using the Soxhlet method for 5 h. The conversion to fatty methyl esters (FAME) and quantification were performed following the methodology of Chirinos et al. (2013). Briefly, the oil was trans-esterified with KOH at 70 °C for 1 h. The samples were run on a GC-FID (Agilent Technologies, Santa Clara, CA, EE.UU), helium was used as 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. The column used corresponded to HP-