Cultivar and tissue-specific changes of abscisic acid, its catabolites and individual sugars during postharvest handling of flat peaches (*Prunus persica* cv. platycarpa)

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Abstract

The role of abscisic acid (ABA) during postharvest ripening of peaches remains unclear. This study aimed to investigate the temporal and tissue-specific changes in ABA, and ABA catabolites, of two flat peaches cultivars, 'Plane Sun' and 'Platibell', during the current stone fruit supply chain. The relationship between ABA catabolism, ethylene production, individual sugar changes and fruit firmness was also studied. We found that flat peaches can produce and metabolise ABA during postharvest ripening, and that this is cultivar and tissue dependent. Our results demonstrated that a burst in ABA concentration preceded that of ethylene production in 'Plane Sun' fruit, suggesting a cross-talk between the two hormones. ABA and ethylene were both negatively correlated with fruit firmness, whilst sugar content, especially glucose, was only relatively correlated with ABA. In conclusion, ABA may trigger ethylene production changes while also affects sugar metabolism leading to fruit softening and over-ripening associated processes during postharvest handling.

Keywords: ethylene, non-structural carbohydrates, ripening control, senescence

Introduction

Understanding fruit ripening and senescence mechanisms behind postharvest behaviour of fresh produce is key to maintain fruit and vegetables nutritional and physiological quality across the supply chain (Falagán and Terry, 2018). Climacteric fruit such as flat peaches (*Prunus persica* L. Batsch cv. *platicarpa* L.H Bailey) are highly perishable and therefore difficult to maintain their properties from farm to fork.

Ethylene and abscisic acid (ABA) are two endogenous hormones known to control the major physiological (e.g., colour, firmness, flavour and aroma) and biochemical changes occurring during stone fruit ripening and senescence (Setha, 2005; Giné-Bordonaba et al., 2016). In climacteric fruits, ethylene is thought be the main driver of fruit ripening (Farcuh et al., 2018; Wang et al., 2019; Lindo-García et al., 2020), while ABA seems to be a major player in the regulation of ripening in non-climacteric fruits, such as strawberries and grapes (Jia et al., 2017; Pérez-Llorca et al., 2019; Bai et al., 2021). Previous studies have elucidated the action of ethylene during preharvest ripening of stone fruit (Huijuan et al., 2018; Shuai et al., 2018). However, the role of ethylene in postharvest ripening and senescence of stone fruit, and especially its relationship with ABA and ABA-catabolites remains unclear.

Not only ABA but rather the balance between ABA biosynthesis and catabolism have been recently suggested to co-ordinately regulate fruit ripening (Liao et al., 2018). ABA-derived phaseic acid (PA), dihydrophaseic acid (DPA), and 7-hydroxy-ABA (7-OH-ABA) are the main ABA catabolites and are generally referred as inactive molecules (Nambara and Marion-Poll, 2005). Nevertheless, PA was earlier described as a molecule

able to activate a subset of ABA receptors yet with a much-reduced activity (*ca*. 10 % of that of ABA) (Weng et al., 2016). Alternatively, ABA can be conjugated to glucose forming the inactive ABA glucose ester (ABA-GE), which is stored or transported (Lee et al., 2006).

In climacteric fruit, ripening starts and accelerates with the burst of autocatalytic ethylene production. It has been observed that ethylene can induce accumulation of ABA at the onset of ripening, suggesting a complex crosstalk between the two hormones (Meyer et al., 2017). However, other studies have suggested an influence of ABA on both preventing over-ripening during cold storage (Tijero et al., 2019) and triggering fruit senescence (Luo et al., 2019).

Furthermore, evidence suggest a cross-talk between ABA and/or ethylene and sugar metabolism as key regulatory hubs of fruit ripening (Jia et al., 2017; Luo et al., 2020). Sugars are not only the main substrate for the fruit respiration, but also possess a central role in fruit ripening and quality because sugar metabolism and accumulation greatly influence taste (Bai et al., 2021). Notably, sucrose has been shown to serve as a signal facilitating strawberry and tomato fruit ripening by stimulating ABA production and accumulation (Jia et al., 2016).

In this study, we investigated if temporal and tissue-specific changes in ABA and its catabolites could have a synergetic action with fruit ethylene production, as well as sugar metabolism, leading to ripening-related changes, including firmness loss, during postharvest handling of two flat peach cultivars.

Material and Methods

Plant material

Two cultivars of flat peaches (*Prunus persica* L. Batsch cv. *platicarpa* L.H Bailey) 'Plane Sun' (yellow flesh) and 'Platibell' (white flesh) were harvested from a commercial orchard in Belver de Cinca (Spain; 41º41'00''N 0º12'50''E). Both cultivars are common within the Spanish market and were selected for the present study based on their distinct flesh colour/carotenoid content. Fruit was first evaluated at the IRTA facilities in Lleida (Spain) and then transported by refrigerated truck (2 days, 2 ºC, 90 % RH) to the Plant Science Laboratory at Cranfield University (UK), simulating current commercial practice.

Experimental design

Flat peaches from both cultivars were subjected to current industry practice conditions for ready to eat or ripen peach fruit within UK [10-15 Newton (N); Crisosto, 1999]. This process involved ripening at room temperature (2 days, 18-22 ºC) immediately after transport and reception (5 days, 2 ºC), followed by a shelf-life period (4 days, 5 ºC), and ending with home life (2 days, 18-22 ºC), as shown in Figure S1. The sampling points were: i) baseline right after harvest (Harvest); ii) after transport; iii) after ripening; iv) at point of sale (retailer); and v) after 2 days of shelf life at the household (home). Three replicates of six individual flat peaches per cultivar (n = 18), at each sampling stage, were analysed. After the physiological assessment, flesh and skin were separately using a sharp stainless-steel peeler, making sure there was minimal mixture in tissues before snap-freezing in liquid nitrogen, freeze-drying and storing at -80 ºC until analysis. The parameters were analysed in two tissues separately (flesh and skin) given the current consumer's preference on whether to eat the stone fruit peeled or not.

Biochemical compounds

Quantification of ABA and ABA catabolites

Freeze-dried powdered flat peach material (5.0 ± 0.1 mg) was weighed and extracted with 500 µL of an ice-cold HPLC grade methanol:water:formic acid (60:35:5 v/v), following Müller and Munné-Bosch (2011) with modifications. The labelled forms of the compounds (-)-5,8´8´8´-d_4-ABA (d4-ABA); (-)-7´,7´,7´-d_3-PA (d3-PA); (-)-7´,7´,7´-d_3-DPA (d3-DPA); (+)-4,5,8´,8´,8´-d_5-ABA-GE (d5-ABA-GE) and (±)-5,8´,8´,8´-d_4-7´-OH-ABA (d4-OH-ABA) were added to the mixture as internal standards at 101 ng mL^{-1}. Endogenous plant growth regulator concentrations were quantified according to Morris et al. (2018) with slight modifications, by using a LC/MS-MS instrument with an Agilent 1200 series HPLC system (Agilent, Berks., UK) coupled to a Q-Trap 6500 mass spectrometer (AB Sciex, Framingham, USA). The extracts were analysed by injecting 20 µL onto a Phenomenex 3 µm C18 Luna 100 x 2 mm with guard column at 40 ºC. The mobile phases were: (A) 2 % acetonitrile in 2 mM ammonium formate, and (B) 95 % acetonitrile in water with 0.1 % formic acid, using an increasing gradient of B (2 % for 4 min, 16 % at 20 min and 34.5 % at 25 min) at a flow rate of 12 mL h^{-1}. A 10-point calibration curve ranging from 0.5 to 3,000 µg L^{-1} was used for quantification. Plant growth regulators concentration was expressed in µg kg^{-1} dry weight (DW).

Individual sugars

Freeze-dried powdered flat peach tissue (150 ± 0.1 mg) were extracted with 3 mL of 62.5:37.5 HPLC grade methanol:water (v/v) and mixed well, according to Foukaraki et al. (2016). Non-structural carbohydrates were quantified using a HPLC binary pump (1200 Series, Agilent, Berks., UK) equipped with an Agilent refractive index detector (G1362A). Flesh and skin extracts (20 µL) were injected into a Rezex RCM monosaccharide Ca^{+} (8 %) size exclusion column of 300 mm x 7.8 mm diameter, 8 µm particle size (Phenomenex, CA; Part no. 00H-0130-K0) with a Carbo-Ca^{2+} security guard

column of 4 mm x 3 mm diameter (Phenomenex, Part no. AJ0-4493). HPLC grade water was used as mobile phase at a flow rate of 0.6 mL min^{-1} (Foukaraki et al., 2014). The presence and abundance of sucrose, glucose, and fructose were calculated by comparing sample peak area to Sigma-Aldrich authentic standards (Dorset, UK). A 9-points calibration curve ranging from 0.025 to 5.00 g L^{-1} was used. Sugar concentrations were expressed as g kg^{-1} DW.

Physiological measurements and quality traits determination

Respiration rate and ethylene production

Respiration rate (RR) was measured according to Collings et al. (2013; 2018a), with modifications, using a Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, Las Vegas, NV, USA). The samples were left *ca.* two hours at room temperature prior to RR measurements. Then, they were placed in 3 L air-tight jars and continuously flashed with dry air at a flow rate of *ca.* 300 mL min^{-1}; the RR (as CO_2 production) was recorded for 2 min per replicate. Cross-contamination between treatment measurements and replicates was prevented using an empty jar (continuous air) as baseline, which was measured for one min. Results were expressed as mg CO_2 kg^{-1} h^{-1} (ExpeData, Release 1.3.8, Version: PRO Software).

Ethylene production was determined using a real-time ethylene analyser with ETD-300 Ethylene detector with CAT-1 catalyser and VC-1 valve control box (Sensor Sense B.V., Netherlands) according to Elmi et al. (2013) and Collings et al. (2018a). Samples were placed into 635 mL air-tight, sealed glass jars which had a continuous flow (4 L h^{-1}) of hydrocarbon free air flushing through (Collings et al., 2018b). An empty jar was recorded at the beginning and end of each run and used as a baseline. Ethylene data was reported

as µL kg^{-1} h^{-1}. Both RR was only measured in the Plant Science laboratory, while the other determinations were also obtained at harvest (IRTA, Lleida, Spain).

Firmness and standard fruit quality parameters at harvest

The firmness of each flat peach was assessed according to Landahl et al. (2010) with slight modifications. An 8 mm flat head probe was mounted onto an Instron Series IX 4301 Universal Testing Machine (Instron, Bucks., UK) fitted with a 500 N load cell. Firmness results were expressed as the average maximum load in N. Total soluble solids, titratable acidity, as well as the Index of absorbance difference (I_{AD}) were measured at harvest as described elsewhere (Giné-Bordonaba et al., 2016; Supplementary Table 1).

Statistics

Statistical analyses were carried out using SPSS Statistics software package v.17.0 for Windows. Analysis of variance (ANOVA) was used to identify significant differences ($p < 0.05$) between the main factors (cultivars and sampling points) and their interactions for each specific fruit tissue (flesh and skin). ANOVA assumptions were tested and found to be valid for this dataset. Least Significant Differences ($LSD_{0.05}$) bars are displayed on graphs or figure legends.

Results

Biochemical response to supply chain conditions

ABA-related metabolism

Changes in ABA during storage were cultivar dependent. ABA was consistently accumulated in flesh and skin tissues of 'Platibell' fruit, throughout the simulated supply chain, being a 9.2 and 7-fold higher at 'home' stage compared to 'harvest', respectively,

for each tissue (Figures 1A and B). However, ABA concentration in 'Plane Sun' fruit reached its maximum after ripening (24,063 and 30,500 µg kg^{-1} in flesh and skin, respectively) to further decrease until the end of the supply chain (10,934 and 13,254 µg kg^{-1}, respectively, in each tissue).

ABA catabolism was also affected by the simulated supply chain. Among the ABA catabolites, DPA was the most abundant in flat peaches, followed by PA (9-fold lower than DPA) and 7-OH-ABA (34-fold lower values than DPA) (Figures 1C and S2). 7-OH-ABA increased after ripening for both cultivars and tissues, and drastically, at home stage in skin tissue (Figures 1C, S2A and B). In addition, the content of 7-OH-ABA along the supply chain was cultivar dependent. After ripening, 'Plane Sun' fruit showed *ca.* 3-fold higher 7-OH-ABA content than 'Platibell' in flesh and skin tissues. Changes of 7-OH-ABA were also tissue dependent, being its content 1.6 and 2.6-fold higher at 'home' stage for 'Platibell' and 'Plane Sun', respectively, in skin than flesh tissues.

ABA-GE was dependant on cultivar and tissue but did not show significant differences throughout the simulated supply chain in flesh tissue, although a significant increase was observed at 'home' stage compared to 'harvest' in skin tissue (Figure 1C, S2C and D). 'Platibell' fruit showed a consistently low concentration across the supply chain in both flesh (mean content of 149 µg kg^{-1}) and skin (mean content of 323 µg kg^{-1}) tissues, while 'Plane Sun' presented an accumulated trend throughout the supply chain, being a 1.6 and 1.9-fold higher at 'home' stage compared to 'harvest' in flesh and skin tissues, respectively. 'Plane Sun' fruit also showed a higher mean content of ABA-GE in skin (14,645 µg kg^{-1}) than flesh (5,300 µg kg^{-1}).

PA and DPA were affected by supply chain stage, cultivar and tissue (Figures 1C, S2E, S2F, S2G and S2H). Both catabolites increased along the simulated supply chain, reaching the highest values at 'retailer' or 'home' stages for both cultivars and tissues.

Along the supply chain, ‘Plane Sun’ fruit showed a significantly higher mean content of PA than ‘Platibell’ fruit in flesh (≈ 1,670 *vs.* 590 µg kg^{-1}) and skin (≈ 4,165 *vs.* 2,436 µg kg^{-1}), being this content 2.5 and 4.1-fold higher in skin than flesh tissue for each cultivar, respectively (Figures 1C, S2E and S2F). In addition, DPA mean content of ‘Plane Sun’ fruit was 1.7 and 1.5-fold higher in flesh and skin tissues, respectively, compared to ‘Platibell’, showing again a 1.2 and 1.4-fold higher content in skin than flesh tissue for each cultivar, respectively (Figure 1C, S2G and S2H). However, these significant differences on PA and DPA content between both cultivars were not observed at ‘home’ stage in skin tissue (Figures 1C, S2E, S2F, S2G and S2H).

Individual sugars

Sucrose was the most abundant sugar in flat peaches, followed by glucose (5.4-fold lower than sucrose) and fructose (5.8-fold lower than sucrose). Glucose and sucrose content significantly decreased by 28 and 20 %, respectively, from ‘harvest’ until ‘after transport’ stage in flesh tissue of ‘Platibell’ fruit (Figures 2A and E). In the same way, ‘Plane Sun’ also showed a significant decrease of 20 and 11 % on glucose and sucrose content, respectively from ‘harvest’ to ‘after transport’ (Figures 2A and 2E).

In contrast, fructose content increased by 23 and 16 % in ‘Platibell’ and ‘Plane Sun’ flesh tissue, respectively, from ‘harvest’ until ‘after ripening’ stage (Figure 2C). Glucose content remained unaffected from ‘after transport’ to ‘home’ stages with average values of 76.86 and 86.99 µg kg^{-1} in ‘Platibell’ and ‘Plane Sun’ flesh, respectively (Figure 2A), while a significant decrease was found in the skin of both cultivars from ‘harvest’ until home life (61 and 76 % for ‘Platibell’ and ‘Plane Sun’, respectively; Figure 2B). In addition, fructose and sucrose in flesh tissue remained constant from ‘after ripening’ until ‘home’ stage for both cultivars (Figure 2C and 2E). With respect to their changes in skin

tissue, fructose content did not show significant differences among sampling stages of supply chain until reaching the simulated 'home' stage, where 'Platibell' and 'Plane Sun' flat peaches showed a significant decrease of 50 and 47 %, respectively (Figure 2D). A significant depletion of sucrose content was also observed throughout the simulated supply chain in skin tissue of both cultivars (Figure 2F), being its mean content significantly higher in 'Platibell' (367.24 g kg^{-1}) than 'Plane Sun' (229.51 g kg^{-1}) fruit at 'retailer' stage (Figure 2F). Finally, sucrose content was higher in flesh (mean of 551 g kg^{-1}) than in the skin (mean of 361 g kg^{-1}) for both cultivars (Figures 2E and 2F). As general trend, a significant drop for all individual sugars was observed at 'home' stage in the skin tissue (Figures 2B, 2D and 2F).

Physiological and quality changes under supply chain conditions

'Plane Sun' flat peaches showed significantly higher RR than 'Platibell' fruit during 'home life' with values of 91.72 *vs*. 67.63 mg CO_2 kg^{-1} h^{-1}, respectively (Figure 3A). Also, whilst 'Platibell' fruit only showed an increase of 29 % in RR during home life, 'Plane Sun' fruit had a previous burst in RR (by 35 %) at 'after ripening' stage, followed by a second peak by 37 % in home life respect to 'retailer' stage (Figure 3A).

Concomitantly, the ethylene response to the simulated supply chain differed between cultivars: 'Platibell' fruit maintained a fairly constant ethylene production (mean of 5.14 µL kg^{-1} h^{-1}) whilst 'Plane Sun' showed a consistent production of ethylene reaching a mean value of 75.96 µL kg^{-1} h^{-1} after 2 days of home life than 'Platibell' (mean of 8.54 µL kg^{-1} h^{-1}) as also observed for the fruit RR (Figures 3A and B).

'Plane Sun' fruit presented similar firmness values compared with 'Platibell' at 'harvest' stage (Figure S3), as for other quality attributes (*viz*. TSS, TA, I_{AD} – Supplementary Table

1). The rate of firmness loss was similar between both cultivars, without significant differences among sampling stages from 'after ripening' stage until home life (Figure S3).

Discussion

ABA plays a role in flat peaches postharvest ripening

Fruits are divided into climacteric and non-climacteric depending on their ability to produce a transient peak in respiration rate and an autocatalytic peak in ethylene production during ripening (Bai et al., 2021). In climacteric species such as peach, ethylene is thought be the main driver of fruit ripening (Wang et al., 2019; Lindo-García et al., 2020); while ABA controls fruit ripening in non-climacteric species such as strawberries and grapes, generally in an ethylene-independent manner (Jia et al., 2017; Pérez-Llorca et al., 2019; Bai et al., 2021). This said, research done over the past decade has clearly demonstrated that ABA is also involved in ripening of tomato (Jia et al., 2016; Bai et al., 2021), pears (Lindo-García et al., 2020) and other climacteric fruit (Wang et al., 2021). In contrast, controversial information (Soto et al., 2013; Liu, 2019) is currently available regarding the putative role of ABA in preventing or promoting peach ripening-related events depending on the fruit developmental stage. Likewise, in other stone fruits, ABA as well as other hormones are known to regulate fruit ripening (Kuhn et al., 2020). However, to the best of our knowledge, no other studies have focused on investigating the role of ABA during postharvest peach ripening.

Our results demonstrated that a peak in ABA accumulation during 'Plane Sun' flat peach ripening preceded that of ethylene production (Figure 3B), suggesting a role for ABA in the regulation of peach ripening/over-ripening via activation of ethylene metabolism. Indeed, similar findings were reported in tomato fruit where exogenous application of ABA promoted fruit ripening through the regulation of transcription factors known to act

as regulators of ethylene synthesis and sensibility (Wu et al., 2018). Wang et al. (2018) also showed that ABA facilitated ethylene production in apples via AREB (ABA responsive element binding protein)/ABF (ABRE binding factors ABA responsive factors)-mediated regulation of genes involved in ethylene biosynthesis (ACS and ACO). In contrast, a recent study on peach fruit suggested that ethylene can regulate the expression of the ABA biosynthetic gene *PpNCED* through specific ethylene response factor *PpERF3* (Wang et al., 2019). Based on our results, and when comparing both cultivars, it seemed that once the ethylene biosynthesis is triggered by ABA, the levels of the later hormone need to decline, as in 'Plane Sun', to further facilitate the ethylene climacteric rise. We therefore suggest that ABA may have a dual effect on peach ripening as previously reported by others (Barry et al., 2000; Soto et al., 2013).

Our data also reveals a strong negative correlation for ethylene production and ABA content with firmness values (Figure S4). Accordingly, ethylene, as well as cold storage, are known to induce the action of specific cell wall degrading enzymes. How ABA can modulate the loss of fruit firmness is yet elusive even though recent evidence suggests that ABA may modulate peach ripening through interference not only with ethylene but also with cell wall related genes (Liu, 2019).

In our study, not only ABA but its catabolites showed a tissue and cultivar-specific temporal pattern during postharvest ripening. This result was not surprising since ABA is *de novo* synthesised from carotenoids, via NCED gene expression, including β-carotene and β-cryptoxanthin, which are the main pigments responsible for the yellow-orange colour of yellow-flesh peach cultivars (Brown et al., 2014). To the best of our knowledge, this is the first study describing the major ABA catabolites in peach fruit during postharvest storage. Among the ABA catabolites, DPA, a biologically inactive compound, was quantitatively the major catabolite accumulated in the flesh and skin of

both cultivars, thereby supporting previous studies carried out in sweet cherries which showed that the cytochrome P450 enzyme ABA 8'-hydroxilase route was the main ABA catabolic pathway (Setha et al., 2005).

PA is considered as an active ABA catabolite because of its action on the activation of a subset of ABA receptors (Weng et al., 2016). However, based on the relative lower concentration of PA found in both tissues, and PA's reduced efficacy, it is unlikely that PA plays a significant role during flat peach ripening/over-ripening. ABA-GE is a reservoir of ABA for the rapid production of active ABA in a compartmentalized fashion (Lee et al., 2006). ABA-GE was found at very low levels for 'Platibell' fruit throughout the different sampling points and both tissue types, whereas 100-fold higher amounts and little variations were detected in 'Plane Sun' (Figure 1C and S2). These results agreed with previous studies (Nambara and Marion-Poll, 2005), reporting that ABA-GE does not always vary in parallel to the change in ABA levels and therefore, suggesting that conjugation may be differentially regulated among different peach cultivars. Hydroxylation at the 7′, 8′, or 9′ positions is also a common step of ABA catabolism; changes in 7-OH-ABA along the supply chain were again cultivar dependent.

Overall, our data suggest that differences in ABA along the supply chain or between cultivars are unlikely explained by differences in ABA catabolism and hence rather regulated at the biosynthetic level. Moreover, the observed accumulation patterns of ABA and ABA catabolites show that flat peaches produce and metabolise noticeable amounts of ABA during postharvest ripening yet with sound differences among both cultivars and tissue types. Similar results were recently reported by Figueroa et al. (2021) in strawberry fruit.

Individual sugars and abscisic acid cross-talk

Scarce information is available about the changes in individual sugars during postharvest handling of stone fruit (Alamar and Terry, 2013), despite being key components of peach quality and consumer acceptance (Borsani et al., 2009; Cirilli et al., 2016). Sugars are not only important flavour components but also considered as major respiratory substrates in peach and other stone fruit (Famiani et al., 2016). Our results showed that the higher RR observed in 'Plane Sun' was not accompanied by a faster depletion of sugars during storage and, therefore, other compounds such as organic acids might account for the primary bulk of respiratory substrates in this fruit upon harvest (Borsani et al., 2009).

'Plane Sun' and 'Platibell' had a similar sugar profile throughout the supply chain, with sucrose being the most abundant sugar, followed by glucose and fructose; hence in agreement with Cirilli et al. (2016). However, sugar changes along the supply chain appeared to be different between tissue types. In other studies, where sugar profile was investigated during on-tree ripening in sweet cherry epicarp and mesocarp tissues (Walker et al., 2011), no differences between tissues were found; suggesting that tissue specific sugar accumulation is species dependent.

The study herein shows that over-ripening differentially affects the carbohydrate profile of flesh and skin tissue. In skin tissue, all three individual sugars significantly decreased at the end of the supply chain ('home'). In contrast, in flesh samples, the greatest sugar decrease occurred at the beginning of the supply chain, between 'harvest' and 'after transport', remaining fairly constant thereafter (Figure 2). Minor changes in sugar content of peach fruit during postharvest ripening at 20 ºC were also reported by Borsani et al. (2009). It may be therefore plausible to speculate that flesh sugar changes during postharvest handling of flat peaches are mediated by a cold-acclimation process.

Our data also indicated that sugar changes during postharvest handling of flat peaches were not strictly dependent on ethylene since no clear associations between ethylene and

sugars were found along the supply chain (Figure S4). Contrastingly, sugar content, and especially glucose, was negatively correlated to ABA content (Figure S4) yet the sound differences between cultivars regarding ABA changes along postharvest handling were not mimicked by differences in any of the individual sugars investigated herein. Future studies are still needed to further corroborate if a complex sugar-ABA cross-talk is involved in regulating peach fruit ripening during postharvest, as recently pointed out in other fruit species (Jia et al., 2016; 2017; Durán-Soria et al., 2020; Luo et al., 2020).

Conclusions

Flat peaches accumulate and metabolise important quantities of ABA during postharvest ripening yet with sound differences between cultivars and even tissues. Such ABA accumulation seems to play a key role in ripening and over-ripening likely by regulating ethylene production, ethylene-related processes and, to some extent, specific sugar changes. Particularly, our results suggest that ABA triggers, firstly, the autocatalytic ethylene rise yet a reduction in ABA accumulation is then required to further enable ripening or over-ripening related processes. Secondly, ABA may also directly affect non-structural carbohydrates changes as well as fruit softening (Figure 4). Both ABA catabolism and sugar accumulation not only differed along the flat-peaches supply chain but also between cultivars and tissues. Studies investigating upstream gene expression levels involved in ABA, ethylene and sugar metabolism are still needed to further elucidate the mechanisms behind postharvest ripening and over-ripening of flat peaches.

Acknowledgements

The authors would like to thank Nick Wing from Univeg UK and Daniel Ridley-Thomas from Sol Fruit Imports Ltd. for providing the fruit; and the Miguel Hernandez University

374 (PDI and PI International Mobility Scholarship [Resolution 0772/18]) for supporting
375 María Emma García Pastor's stay at Cranfield University.

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548 **Figure Legends**

549 **Figure 1.** Abscisic acid (ABA) content (µg kg^{-1}) dry weight of flesh **(A)** and skin **(B)**
550 tissues for 'Platibell' and 'Plane Sun' flat peaches subjected to current practices for the
551 UK supply chain. Sampling stages: harvest, after transport, after ripening, after simulated
552 transit period (retailer), and after 2 days of home life (home). Data represent means of
553 three replicates ± standard error. $LSD_{0.05}$ bars for the interaction cultivar*time point, and
554 each tissue type, are shown. Heatmaps of plant growth regulators concentration **(C)** for
555 'Platibell' and 'Plane Sun' cultivars at every step of their metabolic pathway for two
556 tissues. Colours in the diagram represent the low or high concentration, ranging from
557 green to red, respectively; and numbers below each colour map represent the different
558 sampling points (1 = harvest; 2 = after transport; 3 = after ripening; 4 = at point of sale
559 [retailer]; and 5 = shelf life at the household [home]).

560

561 **Figure 2.** Individual sugars (glucose **[A-B]**, fructose **[C-D]** and sucrose **[E-F])**, as g kg^{-1}
562 concentration dry weight, of flesh and skin tissues for 'Platibell' and 'Plane Sun' flat
563 peaches subjected to current practices for the UK supply chain. Sampling stages: harvest,
564 after transport, after ripening, after simulated transit period (retailer), and after 2 days of
565 home life (home). Data represent means of three replicates ± standard error. No $LSD_{0.05}$
566 bar implies that the interaction cultivar*time point was not significant.

567

568 **Figure 3.** Respiration rate, as CO_2 production (mg kg^{-1} h^{-1}) **(A)**, and ethylene production
569 (µL kg^{-1} h^{-1}) **(B)** for 'Platibell' and 'Plane Sun' flat peaches subjected to current practices
570 for the UK supply chain. Sampling stages: harvest, after transport, after ripening, after
571 simulated transit period (retailer), and after 2 days of home life (home). Data represent

means of three replicates ± standard error. $LSD_{0.05}$ for the interaction cultivar*time point is only shown when such interaction was significant.

Figure 4. General schema of ABA accumulation/changes and its crosstalk with ethylene and non-structural carbohydrates during postharvest handling of flat peaches. During postharvest ripening, ABA may trigger the autocatalytic ethylene rise yet a reduction in ABA accumulation is then required to initiate ethylene dependent over-ripening. Thick black arrows symbolize a synergetic action between ABA and ethylene or sucrose. Dashed arrows symbolised mechanisms described elsewhere. This model is inspired in Durán-Soria et al. (2020) and Kou et al. (2021). ABA: abscisic acid. C_2H_4: ethylene.

583 **Supplementary Figure Legends**

584 **Figure S1.** Diagram displaying the sampling points for the simulated supply chain.
585 Sampling stages investigated were harvest, after transport, after ripening, after simulated
586 transit period (retailer), and after 2 days of home life (home). The firmness
587 threshold/ranges for the different sampling points considered in this work are the
588 following: 60-80 N for 'harvest' and 'after transport' stages; 9-18 N for 'after ripening',
589 'retailer' and 'home' stages.

590

591 **Figure S2.** Supplementary data of heatmap (**Figure 1C**). Plant growth regulators (7-OH-
592 ABA, ABA-GE, PA and DPA) content (µg kg^{-1}) dry weight of flesh **(A, C, E and G)** and
593 skin **(B, D, F and H)** tissues for 'Platibell' and 'Plane Sun' flat peaches subjected to
594 current practices for the UK supply chain. Sampling stages: harvest, after transport, after
595 ripening, after simulated transit period (retailer), and after 2 days of home life (home).
596 Data represent means of three replicates ± standard error. $LSD_{0.05}$ bar for the interaction
597 cultivar*time point for 7-OH-ABA in flesh and skin tissues as well as for PA and DPA
598 in skin tissue are shown. No $LSD_{0.05}$ bar implies that the interaction cultivar*time point
599 was not significant.

600

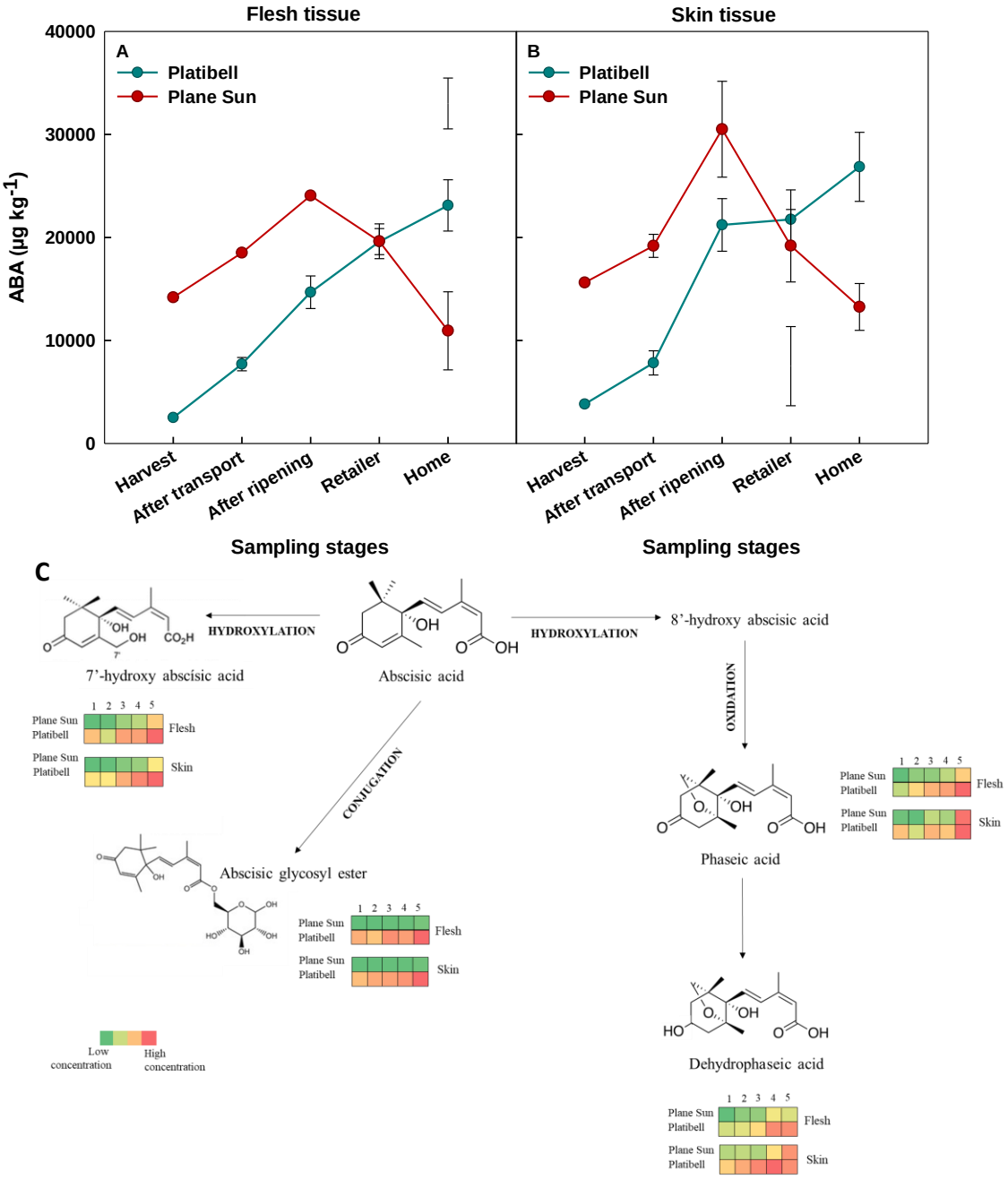
601 **Figure S3.** Firmness (N) for 'Platibell' and 'Plane Sun' flat peaches subjected to current
602 practices for the UK supply chain. Data represent means of three replicates ± standard
603 error. No $LSD_{0.05}$ bar implies that the interaction cultivar*time point was not significant.

604

605 **Figure S4.** Visualization of Spearman's rank correlation matrix between quality and
606 biochemical traits (flesh tissue). Numbers in the diagonal represent the correlations
607 between the same traits among the studied cultivars. Circles above and below the diagonal

608 reported the correlation coefficients between traits for ‘Platibell’ and ‘Plane Sun’,
609 respectively. Colour intensity of each circle is proportional to the correlation coefficients;
610 the circle size is proportional to the significance level; squares denote non-significant
611 correlations ($p > 0.05$).

612 **Figures**



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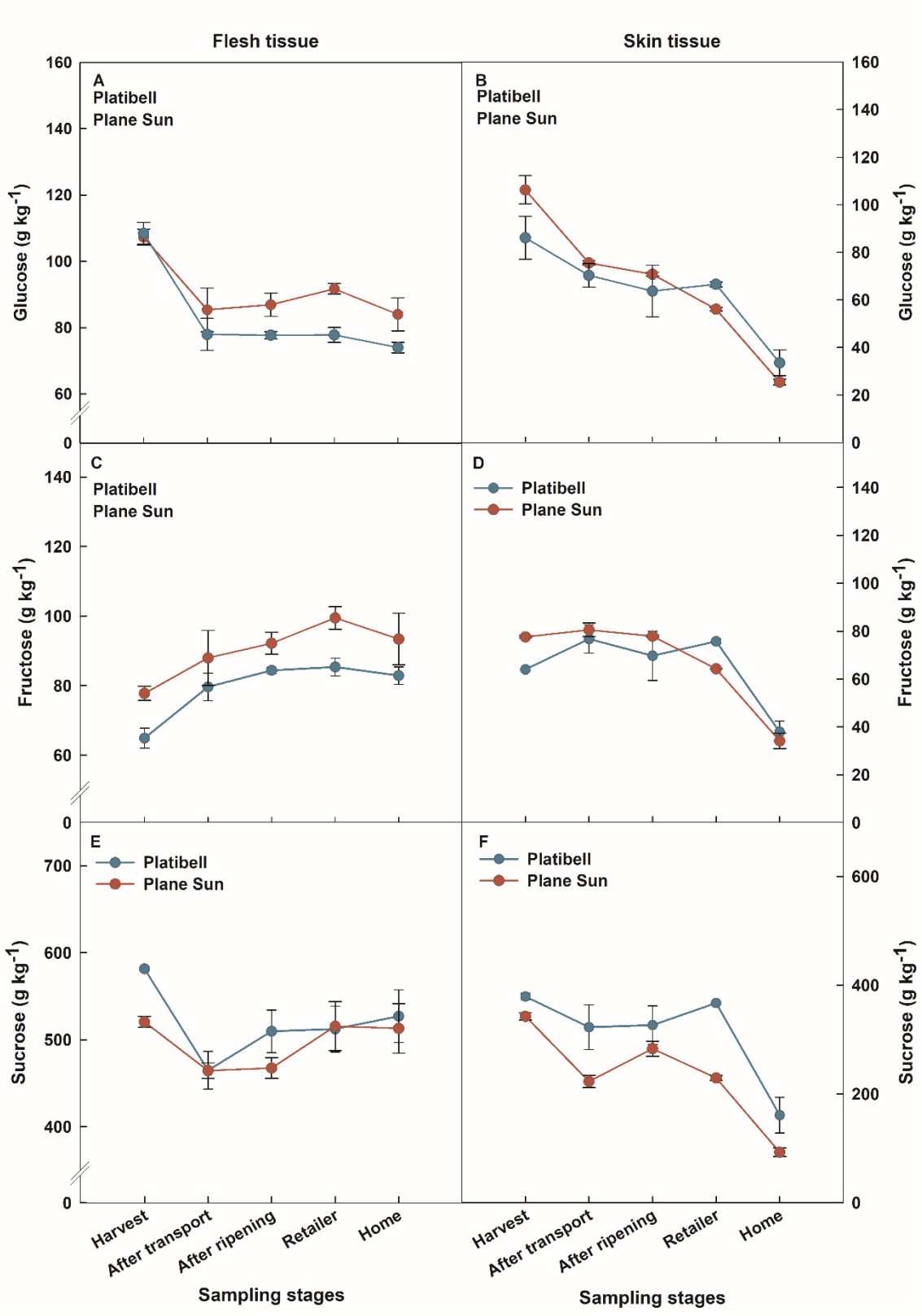
614 **Figure 1.**



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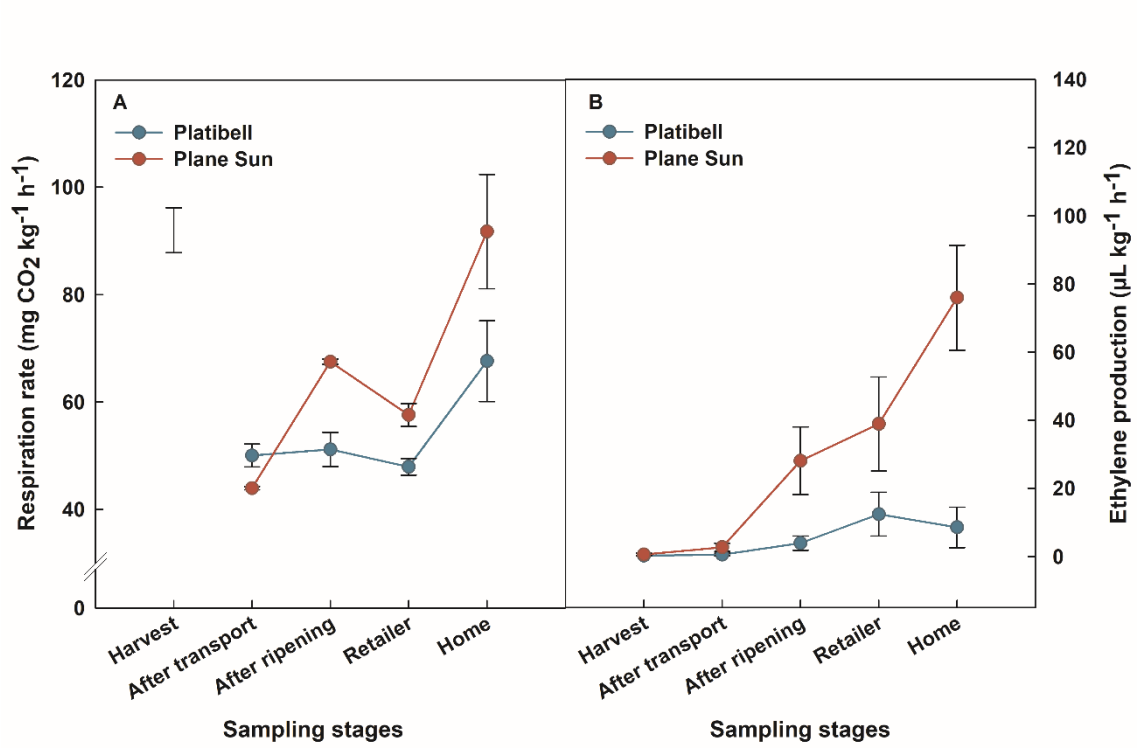

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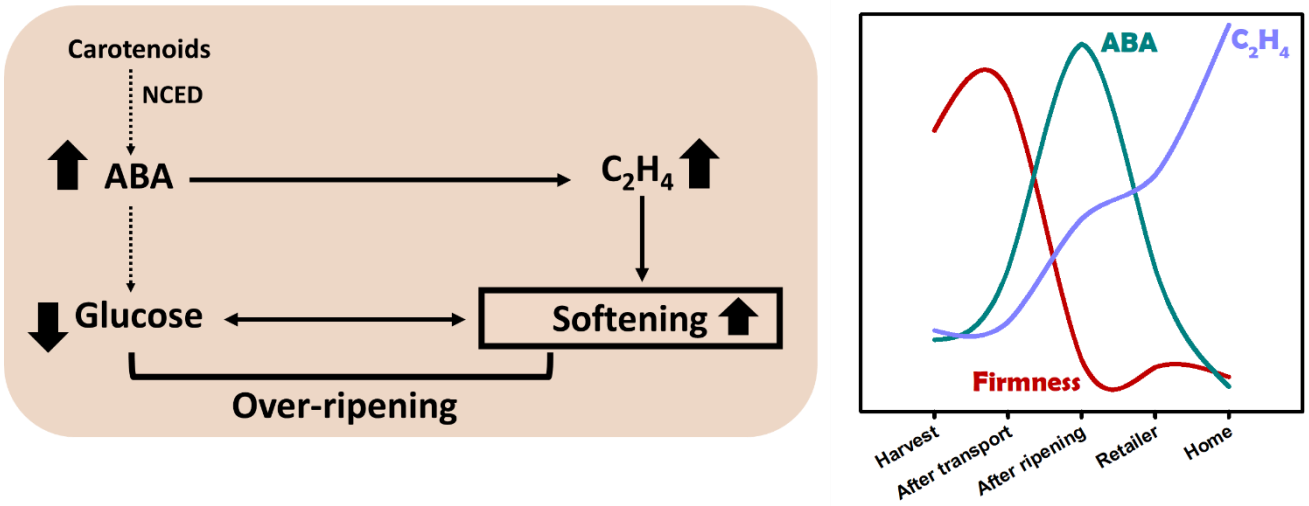

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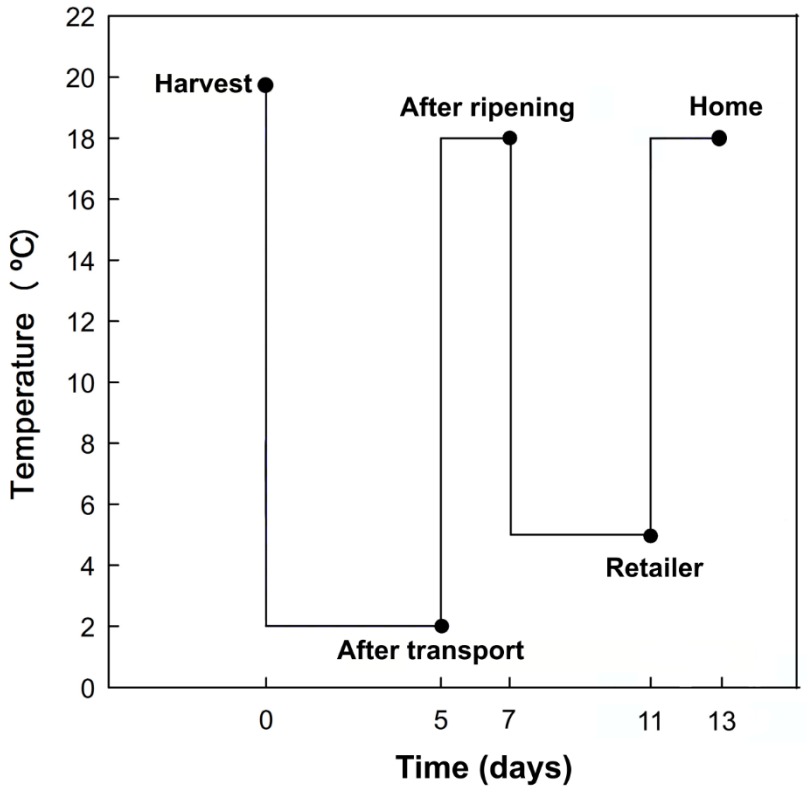

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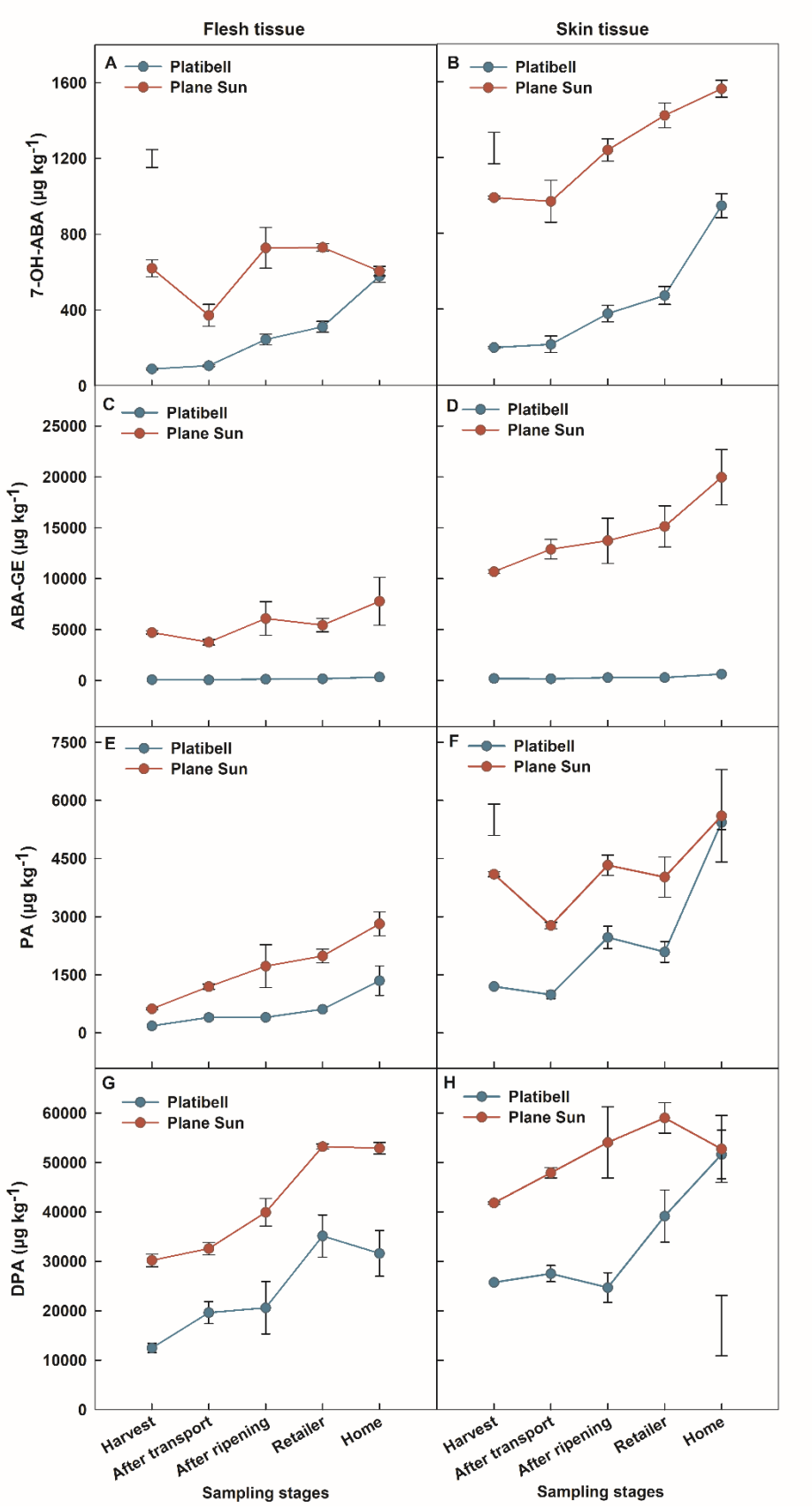
622 **Figure S1**.



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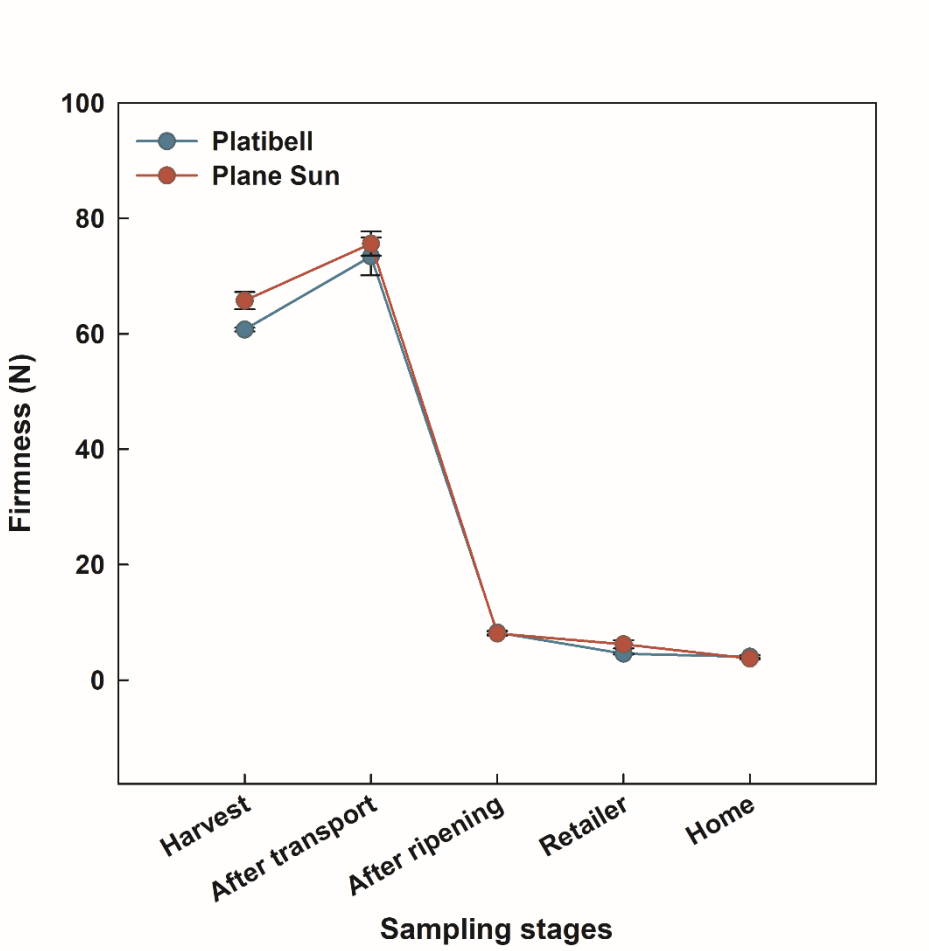
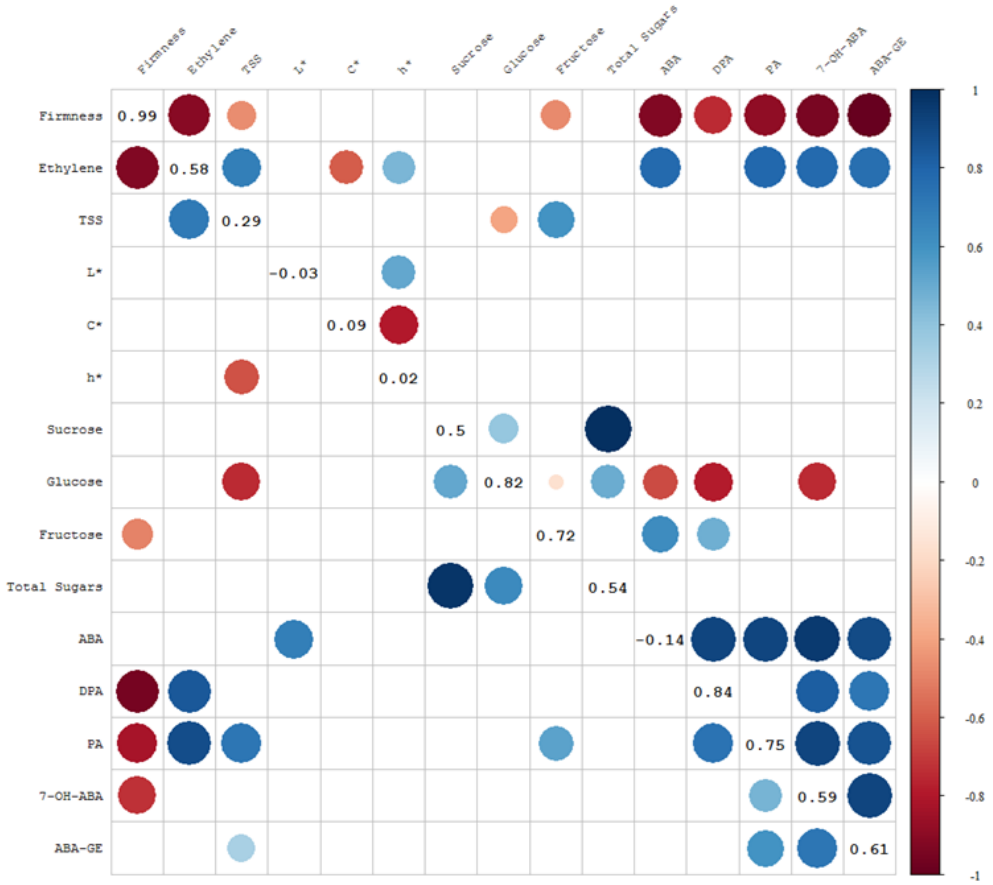
624 **Figure S2.**



625

626 **Figure S3.**

627



628

629 **Figure S4.**

630

631

632 **Supplementary Table 1.** Quality parameters of both flat peach cultivars ('Platibell' and
633 'Plane Sun') at harvest: Index of absorbance difference (I_{AD}), firmness (N), total soluble
634 solids (TSS; º Brix) and total acidity (TA; g NaOH L^{-1}) content.

Cultivar	I_{AD}	Firmness	TSS	TA
Platibell	0.85 ± 0.1	6.2 ± 0.4	12.6 ± 0.2	2.6 ± 0.4
Plane Sun	1.65 ± 0.4	6.5 ± 0.8	12.4 ± 0.1	2.6 ± 0.2

635