Food Control 143 (2023) 109312

Contents lists available at ScienceDirect

Food Control

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

Lipid peroxidation and lipid-soluble antioxidants as quality control markers in cold-stored fruit for establishing commercial acceptability in Bacon avocados

Celia Vincent [a,b], Clara Mirabent [a], Sergi Munné-Bosch [a,b,*]

[a] Department of Evolutionary Biology, Ecology and Environmental Sciences, Spain
[b] Research Institute of Nutrition and Food Safety (INSA), University of Barcelona, Faculty of Biology, Av. Diagonal 643, E-08028, Barcelona, Spain

ARTICLE INFO

Keywords:
Avocado
Chilling injury
Cold storage
Tocochromanols
Carotenoids

ABSTRACT

Avocados are prone to chilling injury disorders when stored at low temperatures. Although most studies have been performed in the worldwide distributed 'Hass' variety, other less exploited commercial varieties such as 'Bacon' might be more cold-tolerant. Aiming at evaluating how cold storage influences chilling injury, the extent of lipid peroxidation and the contents of lipophilic antioxidants were measured in 'Bacon' avocados. Harvested fruit were divided into two groups: one stored for 6 weeks at 4 °C and sampled at various time intervals for 42 days, and the other maintained at low temperatures for 3 and 6 weeks and then exposed to room temperature for 5 days until ripened. Long-term cold storage negatively influenced tocochromanols and carotenoids composition in unripen fruit, decreasing progressively both vitamin E and plastochromanol-8 contents, and most particularly carotenoids; yet chilling injury was prevented during cold storage for 6 weeks. When these fruits were ripened at room temperature, chilling injury increased 20% in the peel but only 5% in the mesocarp (edible part). Enhanced chilling injury in the mesocarp was associated with a 2.3-fold increase in lipid hydroperoxides (a primary lipid peroxidation product) and a 2.5-fold increase in plastochromanol-8 content, while contents of other tocochromanols, carotenoids and malondialdehyde (a secondary product of lipid peroxidation) kept unaltered. Interestingly, medium-term cold storage (3 weeks at 4 °C) did not lead to chilling injury in ripe fruit, therefore cold storage for 3 weeks is considered optimal to prevent large antioxidant vitamin degradation and maintain fruit quality in ripen avocados. In conclusion, despite the high cold tolerance shown by 'Bacon' avocados, fruit showed a strong depletion (60%) in carotenoids content after 6 weeks of storage at 4 °C, negatively affecting their commercial acceptability. Aside from evaluating visual chilling injury symptoms, evaluation of lipid peroxidation markers and contents of lipid-soluble antioxidants are deemed to be essential to establish commercial acceptability in cold-stored fruit.

1. Introduction

Avocados (*Persea americana* Mill.) are fruit with an increasing global economic relevance which are gaining attention of many consumers due to their nutritional and beneficial properties to human health (Araújo et al., 2018). Avocado market has exponentially grown over the last decade corresponding to 600,000 ha and more than 6,000,000 t in cultivation and production, respectively. Avocados are climacteric fruit that ripen very quickly once harvested from the tree (Bower & Cutting, 1988), so that full ripeness is attained in no longer than 5–13 days, depending on the variety (Kassim et al., 2013; Pérez et al., 2004). Thereafter, overripening occurs rapidly and commercial acceptability can be lost due to fruit softening, occurrence of various fungal diseases and a loss of their overall quality (Zhang et al., 2013). However, the antioxidant composition of avocados is rarely considered as an essential component of commercial acceptability in postharvest processing of this climacteric fruit. Postharvest avocado handling overseas can last over one month (Kassim et al., 2013) since several food control strategies have been successfully implemented along the supply chain to guarantee prevention of excessive fruit softening and/or occurrence of various fungal diseases. But again, how long-term cold storage influences overall quality in terms of the antioxidant composition of fruit has been little explored, although antioxidant contents play an essential role in human nutrition and must therefore be considered of high relevance in fruit

* Corresponding author. Department of Evolutionary Biology, Ecology and Environmental Sciences, Spain.
E-mail address: smunne@ub.edu (S. Munné-Bosch).

https://doi.org/10.1016/j.foodcont.2022.109312
Received 2 April 2022; Received in revised form 30 June 2022; Accepted 9 August 2022
Available online 26 August 2022

quality assessments for consumers (Shahidi, 2015).

Although many strategies have been already developed aiming to inhibit fruit ripening in avocados, low temperature storage (between 4 °C and 13 °C, depending on the cultivar) is a very common process implemented for extending avocado shelf life and postharvest quality. Cold storage diminishes metabolic processes, consequently delaying the characteristic respiratory and ethylene peaks occurring during ripening, therefore prolonging fruit shelf life (Bill et al., 2014). Several commercial varieties, like 'Hass' or 'Fuerte', can be stored for 2–4 weeks at 5–7 °C (Yahia & Woolf, 2011). Nonetheless, exposure of avocados at those temperatures over longer periods of time or exposure at temperatures below the critical threshold can affect avocado quality by causing chilling injury (Kassim et al., 2013), which is attributed to great economical postharvest losses, together with mechanical damage, fruit degradation and insect attack (Yahia & Woolf, 2011). Previous studies in 'Hass' avocados have shown that low temperature storage affects fruit firmness and mesocarp discolouration (Zauberman & Jobin-Decor, 1995). Despite cold tolerance has been extensively studied in 'Hass' (Table S1), other commercial varieties have been little explored, and no studies have considered the 'Bacon' variety thus far, despite this is a commercial variety presenting a high tolerance to low temperatures. Hence, 'Bacon' avocado trees, which are highly cultivated mostly in Mediterranean climates, are characteristic of tolerating very low temperatures for longer periods of time than other commercial varieties like 'Hass' or 'Fuerte'. Nonetheless, little attention has been given to cold tolerance in 'Bacon' fruit, what is limiting the improvements of postharvest management of this commercial variety. Although global 'Hass' production nowadays reaches up to 88%, 'Bacon' still represents a relevant percentage of avocado production and its appreciation is increasing for consumers due to the properties that differentiates it from 'Hass' (Table S2, Vincent et al., 2020a).

Tropical and subtropical fruit are prone to cold stress and develop chilling injury when exposed to low temperatures (Jackman et al., 1988). Chilling injury symptoms can appear both in mesocarp (edible part, the pulp) and skin (peel) of the fruit being reflected as pitting and mesocarp decolouration, resulting from oxidative processes (Gómez-López, 2002; Kassim et al., 2013). However, to what extent this chilling injury is associated to lipid peroxidation events in avocados is still poorly understood. Stress conditions like low-temperature storage generally cause a rise in reactive oxygen species (ROS) that induce oxidative stress and may eventually trigger lipid peroxidation processes in plant cells (Gill & Tuteja, 2010). As a result, cell disruption and loss of fruit integrity is expected to occur if the stress is sustained, probably attributed to enhanced lipid peroxidation events and/or a drop in antioxidant contents (Shewfelt & del Rosario, 2000; Prabath Pathirana et al., 2013). In this respect, lipophilic antioxidants like tocopherols, tocotrienols, plastochromanol-8 and carotenoids play essential roles in preventing lipid peroxidation, most particularly α-tocopherol, which is described to be the most active form of tocochromanols and the only lipid-soluble antioxidant universally occurring in plants with a chain-breaking antioxidant capacity (Falk & Munné-Bosch, 2010).

Here, we aimed at better understanding how low temperatures in the cold-tolerant variety 'Bacon' avocado affects antioxidant and physiological quality when stored for long periods of time. Since chilling injury in this fruit is reported to have a high economic impact in European agriculture and exportation (Bill et al., 2014), the present study is of great interest to establish the most adequate postharvest management strategies in this still poorly studied cold-tolerant variety of avocados.

2. Material and methods

2.1. Plant material and sampling

Mature but still unripe avocados (*Persea americana* Mill. cv. Bacon) with a dry matter value of 21% were harvested from a commercial orchard in Málaga (south Spain) and immediately brought to the laboratory at the University of Barcelona (Barcelona, NE Spain) after 12 h of transportation at 8–10 °C. Upon arrival, fruit were selected for homogeneity according to their size, maturation stage and lack of pathogen symptoms. Then, avocados were divided into two groups. The first group was stored at 4 °C in a cold chamber up to 42 days (6 weeks, relative humidity of 58 ± 2%) in order to study long term cold-tolerance, and samplings were performed at 0, 7, 10, 14, 20, 21, 28, 30, 34, 40 and 42 days of cold storage. A second group was stored at 4 °C in a cold chamber but just upon arrival (time 0 weeks) and after 3 and 6 weeks of cold storage, fruit was ripened at room temperature (20 ± 3 °C) and relative humidity (60 ± 2%) for 5 days to evaluate the effects of ripening on fruit quality, as well as its dependence on previous time of cold storage. For all experiments, fruit were always kept in darkness throughout the study periods. For each sampling time, avocados were weighed and cut in halves to subsequently attribute a value of mesocarp discoloration to each half according to the percentage of area affected in relation to chilling injury. To follow, the peel and stone were removed from the other half of the fruit and mesocarp tissue was cut into pieces and immediately frozen in liquid nitrogen and kept at −80 °C until analyses.

2.2. Fruit firmness

The stage of ripening of fruit was assessed by manually measuring firmness with a penetrometer (model FT 327, QA Supplies, Norfolk, VA, USA) with a 6×10^{-3} m cone following manufacturer instructions. The measurement consisted in pushing the cone of the penetrometer from a distance of 3×10^{-2} m into the fruit, avoiding the contact with the stone.

2.3. Lipid peroxidation analyses

Lipid peroxidation levels were determined by analysing primary (lipid hydroperoxide) and secondary (malondialdehyde) lipid peroxidation products. First, for evaluating lipid hydroperoxides content, frozen samples were extracted with methanol containing 0.01% butylhydroxytoluene (BHT; w/v) at 4 °C using 30 min of ultrasonication at a potency of 110 W (Bransonic ultrasonic bath 2800, Emerson Industrial, Danbury, CT, USA) and centrifugation at 1419 × g for 10 min at 4 °C. Then, two re-extractions were performed. Supernatants were pooled and used for analyses using the Fox-2 reagent (consisting in a solution of 90% methanol [v/v] containing 25 mM sulfuric acid, 4 mM BHT, 0.25 mM iron sulphate ammonium (II) and 0.1 mM xylenol orange) as described in Bou et al. (2008). Absorbances were measured at 560 and 800 nm. A calibration curve using hydrogen peroxide at various concentrations was used for quantification.

For determining the malondialdehyde content, the thiobarbituric acid-reactive substances assay, was used as in Hodges et al. (1999). In brief, samples were extracted with 80% (v/v) ethanol containing 0.01% (w/v) BHT, then vortexed for 20 s and exposed to ultrasonication for 45 min at a potency of 110 W (Bransonic ultrasonic bath 2800). After a centrifugation process at room temperature for 13 min at 1091 × g, the supernatant was recovered, and the pellet re-extracted twice using the same procedure. Then, two aliquots for sample were used: (a) -TBA, with 1 mL extract + 1 mL 20% trichloroacetic acid (w/v) with 0.01% BHT (w/v) and (b) +TBA, with 1 mL extract + 1 mL 20% trichloroacetic acid (w/v), 0.01% BHT (w/v) and 0.65% thiobarbutiric acid (w/v). Tubes were incubated at 95 °C for 25 min. Subsequently, the reaction was stopped by maintaining them at 4 °C for 10 min. After centrifugation at 1091 × g at room temperature for 5 min, malondialdehyde content in samples were analysed by spectrophotometry and quantified using the equations developed by Hodges et al. (1999).

2.4. Tocochromanols analyses

The quantification of the different forms of tocochromanols, including tocopherols, tocotrienols and plastochromanol-8 was

performed as follows. Samples were extracted with methanol containing 0,01% BHT (w/v) using vortex for 20 s followed by 30 min of ultrasonication at a potency of 110W (Bransonic ultrasonic bath 2800). Pellets were then re-extracted twice following the same procedure. Then, supernatants were pooled and centrifuged at 1419 × g during 10 min at 4 °C before passing them onto a hydrophobic 0.22 μm PTFE filter (Phenomenex, Torrance, CA, USA) and introducing them into vials. Tocochromanols were separated by HPLC using an Inertsil 100A column (5 μm, 0.03 × 0.25 m, GL Sciences Inc., Japan). A Jasco fluorescence detector (FP-1520, Tokyo, Japan) and a calibration curve established with authentic standards (Sigma) for each of the tocochromanols analysed were used for quantification.

2.5. Chlorophylls and carotenoids

To determine total chlorophyll and carotenoids content, 100 mg mesocarp sample were extracted in 1 mL of methanol +0.01% BHT using ultrasonication, vortex and centrifugation, as explained in the previous section. Supernatants were collected and centrifuged for 10 min at 1419 × *g* at 4 °C (Hettich Universal 32R centrifuge, Thermo Fischer Scientific). Chlorophylls and carotenoids were measured spectrophotometrically by reading absorbances at 470, 653, 666 and 750 nm (modified from Wang et al., 2010 and Lichtenthaler & Buschmann, 2001) as follows:

Chlorophyll *a* (Chl a) = [12.21 x (A_{666} – A_{750})] – [2.81 x (A_{653} – A_{750})]

Chlorophyll *b* (Chl b) = [20.13 x (A_{653} – A_{750})] – [5.03 x (A_{666} – A_{750})]

Carotenoids = [(1000 x A_{470}) – (1.63 x A_{653}) – (104.96 x A_{666})] / 221

2.6. Determination of chilling injury

In avocado halves, chilling injury was visually estimated as the percentage of affection on fruit according to mesocarp discolouration. Then, a percentage of affection was attributed to fruits for each sampling time. Skin chilling injury was measured similarly considering external black patches as the main affection of the disorder in 'Bacon' avocado peel.

2.7. Statistical analyses

Statistical analyses were performed by applying an one-way ANOVA for the first experiment (cold storage effects) with 6 replicates in each sampling time point, except time 0 which was measured using 12 replicates (IBS SPSS Statistics 19; SPSS Inc., Illinois, USA). For the second experiment (ripening effects on cold-stored fruit), two-way ANOVA and Tukey *posthoc* tests were applied in a number of 12 replicates, indicating with asterisks when ripe fruit differed from unripe fruit for each sampling time. Differences between ripe and unripe fruit were considered significant when p values were below 0.05 ($p < 0.05$). All data are presented on a dry weight basis.

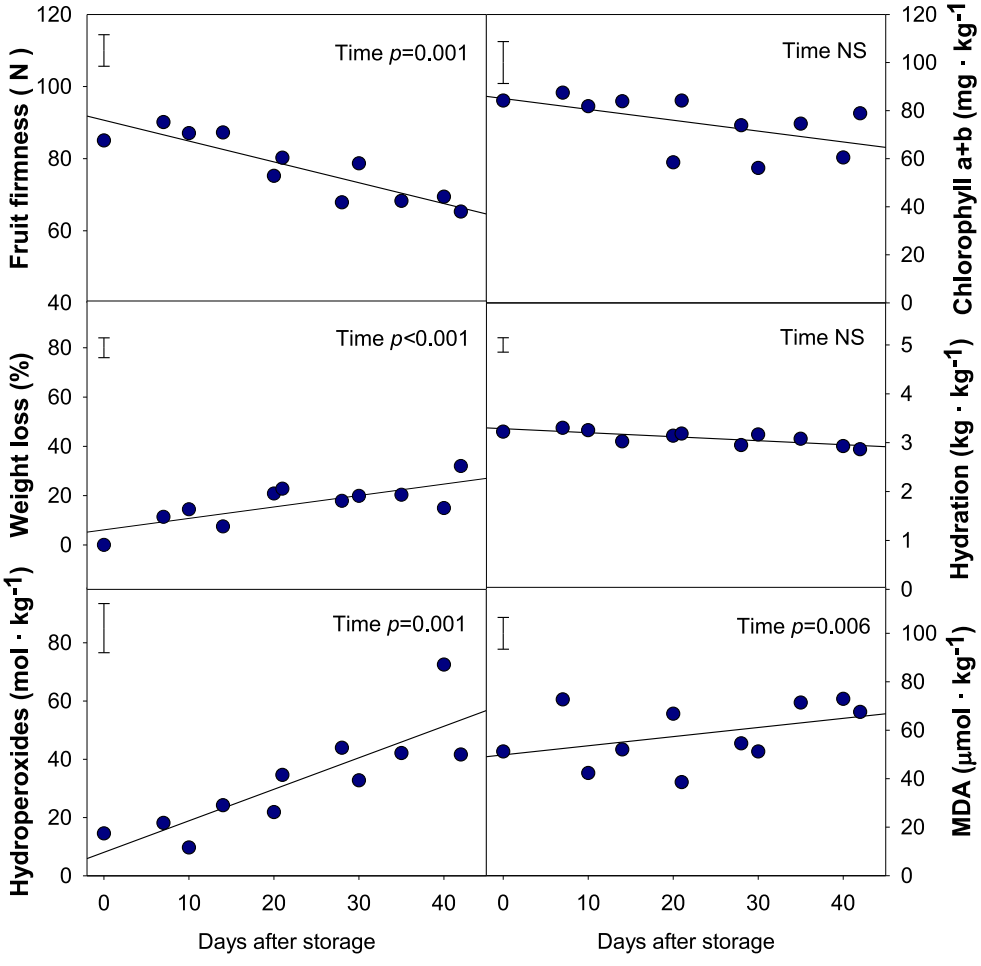

Fig. 1. Time-course variations in fruit quality, as indicated by fruit firmness, total chlorophyll, biomass, hydration, hydroperoxides and malondialdehyde content in 'Bacon' avocados stored for 42 days at 4 °C. Data show the mean of n = 6 fruits for each sampling point, except for time 0 days when n = 12. A global standard error is indicated as a measurement of the variability obtained for each parameter in the inlet as the mean of standard errors obtained in all time points. Data were fitted with a linear regression. Results of one-way ANOVAs are shown in the inlets. MDA, malondialdehyde.

3. Results

3.1. Cold storage effects on fruit firmness and antioxidant quality

Long term storage influenced physiological parameters on avocado mesocarp causing a 23% and 33% reduction in fruit firmness and weight, respectively, which was not related to water losses according to hydration values that did not vary over time (Fig. 1). Lipid peroxidation events increased significantly with cold storage, as indicated by enhanced contents of primary products (hydroperoxides) and secondary products (malondialdehyde) of lipid peroxidation. However, these oxidative reactions did not negatively affect total chlorophyll contents (Fig. 1). Furthermore, chilling injury symptoms did not increase significantly upon 42 days of storage at low temperatures, neither in the skin (11.67 ± 8.67% at 42 days *vs.* 0% at the start of the experiment; $p = 0.176$) nor in the mesocarp (with undetectable injury symptoms both at the beginning and end of the experiment, data not shown).

Long-term cold storage decreased progressively both tocochromanols and carotenoids contents (Fig. 2), fruit mesocarp losing antioxidant capacity and reducing its activity against lipid peroxidation. Total vitamin E levels decreased mainly due to reductions of 11% in α-tocopherol contents, while contents of both γ-tocopherol and plastochromanol-8 decreased to a lesser extent. γ-Tocotrienol content kept unaltered along the cold storage (Fig. 2). In contrast, carotenoid content decreased sharply during 42 days of cold storage, showing a 60% reduction compared to initial levels, and being therefore the most degraded compounds. Correlation studies performed to examine possible relationships between antioxidant contents and the extent of lipid peroxidation (Table 1) revealed that α-tocopherol was the antioxidant that most explains primary lipid peroxidation events. By contrast, secondary lipid peroxidation events (indicated here by malondialdehyde accumulation) did not show any significant correlation with the lipophilic antioxidants studied (Table 1).

3.2. Ripening increases chilling injury in long-term stored fruit

Avocado consumption requires ripening to trigger acceptable fruit softening, therefore adapting the fruit to consumer preferences. Although storage at low temperatures can cause uneven ripening in

Table 1
Spearman correlations between lipophilic antioxidants and primary and secondary products of lipid peroxidation in avocado mesocarp.

	Hydroperoxides	Malondialdehyde
Carotenoids	σ −0,499**	σ = −0,081
α-Tocopherol	σ = −0,538**	σ = −0,122
γ-Tocopherol	σ = −0,328**	σ = −0,214
γ-Tocotrienol	σ = −0,009	σ = 0,125
Plastochromanol-8	σ = −0,174	σ = 0,086
Total vitamin E	σ = −0,537	σ = −0,115

Two asterisks indicate corrected p-value ≤0.006.

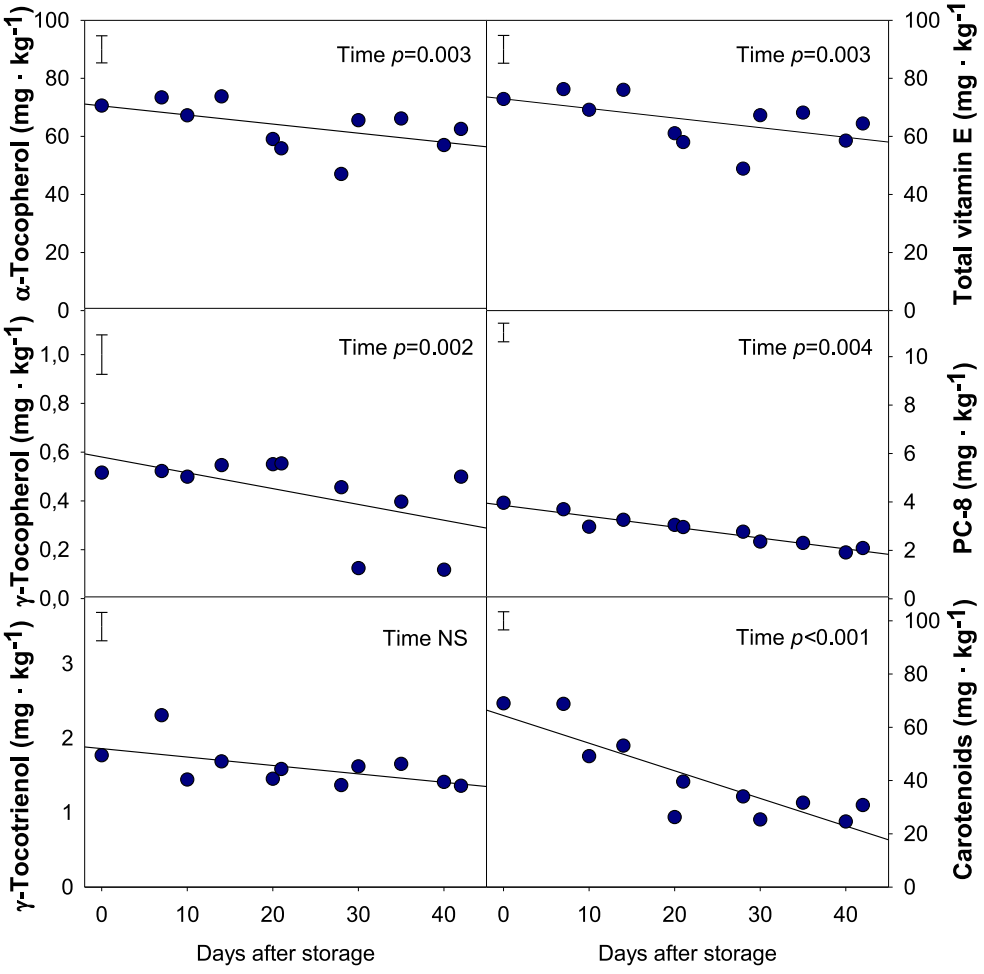

Fig. 2. Time-course variations in the contents of tocopherols, tocotrienols, plastochromanol-8 and carotenoids in 'Bacon' avocados stored for 42 days at 4 °C. Data show the mean of n = 6 fruits for each sampling point, except for time 0 days when n = 12. A global standard error is indicated as a measurement of the variability obtained for each parameter in the inlet as the mean of standard errors obtained in all time points. Data were fitted with a linear regression. Results of one-way ANOVAs are shown in the inlets.

several avocado varieties, 'Bacon' avocados stored at 4 °C for 6 weeks did not present major alterations in this respect. A similar degree of fruit softening was attained in long-term (6 weeks) cold-stored fruit, medium-term (3 weeks) cold stored fruit and non-cold stored fruit, 6 weeks of cold storage guaranteeing a good texture quality for consumers (Fig. 3). Avocado biomass was slightly reduced in ripe fruit, but this weight loss seemed not to be related to water losses as reflected on hydration values. Lipid peroxidation events were triggered in the mesocarp of ripe fruit, as indicated by enhanced lipid hydroperoxides content. Malondialdehyde levels tended to increase in ripe *vs*. unripe fruit, as indicated by results of two-way ANOVA, but did not differ significantly at any time point, as indicated by *posthoc* analyses (Fig. 3). However, these primary lipid peroxidation events did not negatively influence chlorophylls contents (Fig. 3). In contrast, chilling injury incidence occurred in ripen fruit after 6 weeks of storage at 4 °C, despite symptoms attained up to 20% of affection in the skin and only 5% in the mesocarp (edible part of the fruit, Fig. 3). Ripening only slightly influenced tocochromanols content, except for plastochromanol-8, which levels increased 2.5-fold in ripen compared to unripen fruit after 6 weeks of cold storage (Fig. 4). Carotenoid accumulation did not differ between ripe and unripe avocados

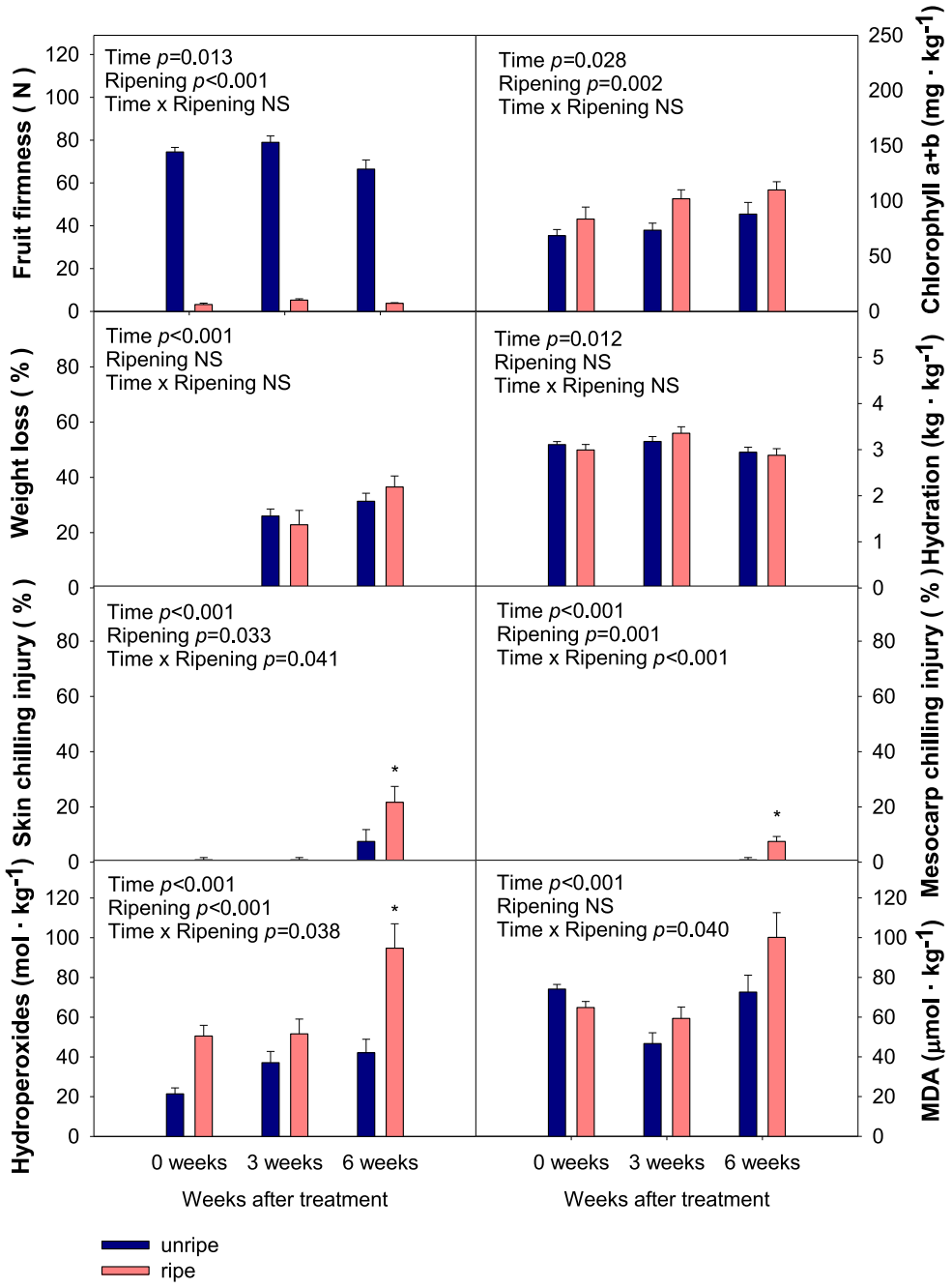

Fig. 3. Fruit firmness, total chlorophyll, fruit biomass, hydration, hydroperoxides and malondialdehyde contents in ripen and unripen avocados stored for 0, 3 and 6 weeks at 4 °C. Ripening was promoted by exposing fruit to 20 °C for 5 days. Data show the mean and standard error of n = 12 fruits. Results of two-way ANOVAs are shown in the inlets. Asterisks indicate significant differences ($p < 0.05$) between unripe and ripe fruit per sampling time. MDA, malondialdehyde.

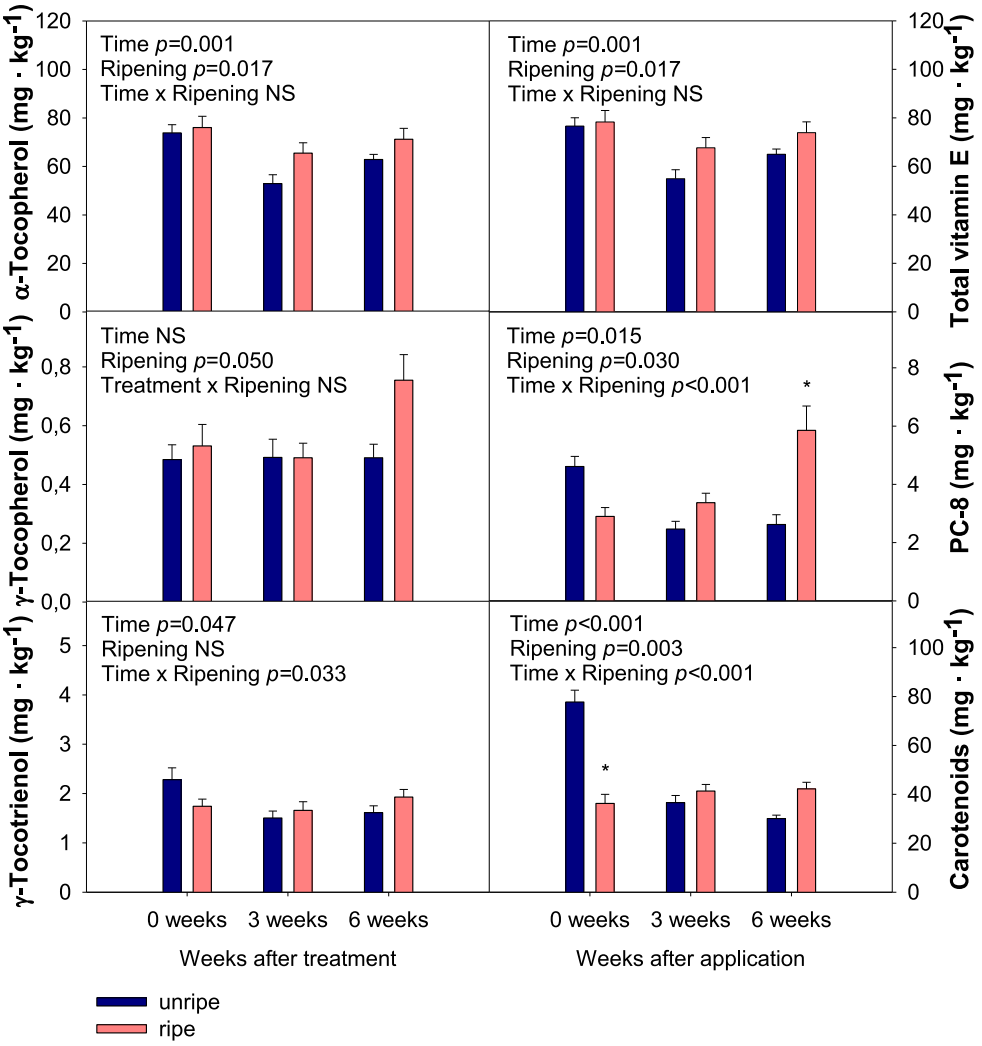

Fig. 4. Tocopherols, tocotrienols, plastochromanol-8 and carotenoids contents in ripen and unripen avocados stored for 0, 3 and 6 weeks at 4 °C. Ripening was promoted by exposing fruit to 20 °C for 5 days. Data show the mean and standard error of n = 12 fruits. Results of two-way ANOVAs are shown in the inlets. Asterisks indicate significant differences ($p < 0.05$) between unripe and ripe fruit per sampling time.

during cold storage, except for a 55% reduction in carotenoid contents in ripe compared to unripe fruit at the beginning of the experiment (Fig. 4). It is interesting to note that ripening had a similarly negative effect on carotenoid contents at 0 weeks (Fig. 4) as 42 days of cold storage in unripen fruit (Fig. 2).

4. Discussion

Fruit quality control during postharvest cold storage is very important to evaluate avocados shelf life. Nevertheless, the threshold of tolerance to low temperatures are highly variable between fruits and varieties and both determine the conditions under which fruit are susceptible to lose a significant quality. Therefore, evaluating the implications of cold storage in various avocado varieties, beyond the highly studied 'Hass' avocado, is of paramount importance, most particularly in cold-tolerant varieties such as 'Bacon', so that we can get a better understanding of tolerance mechanisms and eventually improve postharvest management to guarantee fruit quality.

Stress responses have been associated to oxidative processes in fruit, either related to lipid peroxidation events tiggering signalling responses or degradative processes, the latter particularly occurring when the antioxidant capacity is overwhelmed by pro-oxidant events (He & Ding, 2020; Muñoz & Munné-Bosch, 2018). The extent of lipid peroxidation is often evaluated with the measurement of primary and secondary products of lipid peroxidation, including among others, lipid hydroperoxides and malondialdehyde contents. Our results showed that lipid peroxidation events associated to cold storage were reflected by enhanced hydroperoxides rather than increased malondialdehyde contents, therefore lipid peroxides could be used as an excellent indicator of cold exposure and tolerance in avocados. Interestingly, long-term cold storage in 'Bacon' avocados did not induce chilling injury in the mesocarp for as long as 42 days in unripe fruits. Interestingly, other parameters like firmness or fruit weight were slightly reduced over storage in unripe fruit, although this reduction in fruit weight seemed not to be related to dehydration. Rather, respiratory losses might explain these biomass losses, since avocados are characterized for being highly climacteric fruit with a quite pronounced respiratory rate even when stored at low temperatures (Olivares et al., 2020). Oxidative processes related to lipid peroxidation might have occurred in parallel with a severe depletion on lipophilic antioxidants, mainly due to carotenoids, α-tocopherol and plastochromanol-8 contents in cold-stored fruit. Among these, carotenoids have been reported to exert an important scavenging function

against singlet oxygen, which could otherwise lead to severe lipid peroxidation (Ramel et al., 2012). Similarly, α-tocopherol (which is the tocochromanol with the highest vitamin E activity) and plastochromanol-8 (with a longer hydrophobic-prenyl chain than tocopherols) might equally avoid lipid peroxidation through the elimination of singlet oxygen, but at the same time both could prevent the propagation of lipid peroxidation (Kruk et al., 2014), a function that is not exerted by carotenoids. Therefore, the combined action of all lipophilic antioxidants, like carotenoids, tocopherols and plastochromanol-8 may play a major role in preventing more severe lipid peroxidation events and eventual cell disruption in this highly low-temperature tolerant variety. Notably, the major protective effect as an antioxidant against lipid peroxidation was observed for α-tocopherol, which better correlated with hydroperoxides, but carotenoids and plastochromanol-8 activity should not be underestimated since the progressive decrease induced by the stress suggests a remarkable implication of these compounds. Furthermore, it is worth mentioning that cold storage affects antioxidant composition in avocado mesocarp, therefore inducing a loss of antioxidant capacity against lipid peroxidation and a reduction on fruit quality when stored for 42 days at 4 °C, being of 11% for α-tocopherol, 47% for plastochromanol-8 and 60% for carotenoids, therefore negatively impacting nutritional properties and consumer acceptance. According to previous studies on cold storage influencing vitamin E contents in the same avocado variety, a decrease on α-tocopherol was detected after 10 days of storage at 4 °C (Vincent et al., 2020a) and possible low temperatures effects on vitamin E in a longer term were eclipsed by a positive effect of immersion of pyridoxal 5′-phosphate (Vincent & Munné-Bosch, 2022). Therefore, the present results conclusively show that vitamin E and plastochromanol-8 contents are progressively and negatively affected by long term cold storage (up to 42 days).

Ripening is closely associated to oxidative events related to lipid peroxidation events acting both as a source of signalling molecules and degradative processes (Muñoz & Munné-Bosch, 2018). Ripening of fruit stored for 6 weeks at 4 °C led to enhanced lipid peroxidation, as indicated by increased lipid peroxide contents, as well as increased plastochromanol-8 accumulation. Although the positive role of carotenoids and α-tocopherol is very clear in terms of human health and nutrition (Kan et al., 2022; Power et al., 2022; Weber et al., 1997), the possible benefits of plastochromanol-8 still remain unclear and, taking into account the low values of this antioxidant present in avocados compared to those of α-tocopherol, it may be inferred that the observed increase of plastochromanol-8 in ripe fruit after long-term cold storage may not be of high biological relevance relative to human health. Results obtained in terms of keeping antioxidant contents during ripening are not surprising, since ripening and fruit softening are not generally related to a loss on antioxidant capacity or to chloroplast dismantling in fruit tissues, but rather to cell wall modifications occurring during this physiological process triggered by signalling responses mediated by phytohormones (Fernández-Cancelo et al., 2022; Vincent et al., 2020b).

Noteworthy, fruit quality can be attributed not only to biochemical properties but also to physiological aspects. In this respect, evaluation of chilling injury symptoms is very helpful to examine cold storage effects on market acceptability. On the one hand, storage for 6 weeks had no effect on the development of chilling injury, except when fruits were subsequently left to ripe at room temperature. Even in this case, the edible part of the fruit was little affected (5%), although it is also true that market acceptability decreased too much when considering the skin (20%). As reflected on hydroperoxides contents in fruit manifesting chilling injury, lipid peroxidation may be influencing this disorder, as indicated by enhanced hydroperoxide contents. Therefore, hydroperoxides could be a great indicator of cold stress and chilling injury (performing better than malondialdehyde). Altogether, ripening did not further negatively influence antioxidant quality in cold-stored fruit, but negatively affected quality in terms of chilling injury.

5. Conclusion

In conclusion, 'Bacon' avocados present a high tolerance to low temperatures, but long-term storage causes lipid peroxidation and a loss of lipophilic antioxidants, mainly of carotenoids, which showed a strong depletion (60%), and to a lesser extent of tocochromanols (including tocopherols, tocotrienols and plastochromanol-8), therefore negatively affecting their commercial acceptability. Furthermore, storage for 6 weeks at 4 °C causes the appearance of chilling injury symptoms in ripe fruits, despite negative effects in the mesocarp are low (5%). Introducing measurements of lipophilic antioxidants contents and lipid peroxidation markers, aside from evaluating chilling injury symptoms, are essential if we aim at establishing adequate control management strategies for maintaining fruit quality in cold-stored 'Bacon' avocados. This has important implications in establishing this variety further into the market, while optimizing postharvest strategies to keep adequate avocado quality in terms of human nutrition.

Funding

This work was supported by the Catalan Government through an ICREA Academia award given to S.M.-B.

CRediT authorship contribution statement

Celia Vincent: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, preparation, Writing – review & editing, Visualization. **Clara Mirabent:** Methodology, Investigation, All authors have read and agreed to the published version of the manuscript. **Sergi Munné-Bosch:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft, preparation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

None declared.

Data availability

Data will be made available on request.

Acknowledgements

We want to thank Esther Miralles (CCiT, University of Barcelona) for her technical assistance during HPLC analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.109312.

References

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., & Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science & Technology, 80*, 51–60. https://doi.org/10.1016/j.tifs.2018.07.027

Bill, M., Sivakumar, D., Keith, A., & Korsten, L. (2014). Avocado fruit quality management during the postharvest supply chain. *Food Reviews International, 30*, 169–202. https://doi.org/10.1080/87559129.2014.907304

Bou, R., Codony, R., Tres, A., Decker, E. A., & Guardiola, F. (2008). Determination of hydroperoxides in foods and biological samples by the ferrous oxidation-xylenol orange method: A review of the factors that influence the method's performance. *Analytical Biochemistry, 377*, 1–15. https://doi.org/10.1016/j.ab.2008.02.029

Bower, J. P., & Cutting, J. C. (1988). Avocado fruit development and ripening physiology. In A, & J. Janick (Eds.), *Vol. 10. Horticultural reviews* (pp. 229–261). Portland, OR, USA: Timber Press. https://doi.org/10.1002/9781118060834.ch7, 9781118060834.

Falk, J., & Munné-Bosch, S. (2010). Tocochromanol functions in plants: Antioxidation and beyond. *Journal of Experimental Botany, 61*, 1549–1566. https://doi.org/10.1093/jxb/erq030

Fernández-Cancelo, P., Muñoz, P., Echeverría, G., Larrigaudière, C., Teixidó, N., Munné-Bosch, S., & Giné-Bordonaba, J. (2022). Ethylene and abscisic acid play a key role in modulating apple ripenig after harvest and after cold-storage. *Postharvest Biology and Technology, 188*, Article 111902. https://doi.org/10.1016/j.postharvbio.2022.111902

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry, 48*, 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gómez-López, V. M. (2002). Some biochemical properties of polyphenol oxidase from two varieties of avocado. *Food Chemistry, 77*, 163–169. https://doi.org/10.1016/S0308-8146(01)00331-4

He, M., & Ding, N.-Z. (2020). Plant unsaturated fatty acids: Multiple roles in stress response. *Frontiers of Plant Science, 11*, Article 562785. https://doi.org/10.3389/fpls.2020.562785

Hodges, D. M., DeLong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta, 207*, 604–611.

Jackman, R. L., Yada, R. Y., Marangoni, A., Parkin, K. L., & Stanley, D. W. (1988). Chilling injury. A review of quality aspects. *Journal of Food Quality, 11*, 253–278. https://doi.org/10.1111/j.1745-4557.1988.tb00887.x

Kan, B., Guo, D., Yuan, B., Vuong, A. M., Jiang, D., Zhang, M., Cheng, H., Zhao, Q., Li, B., Feng, L., Huang, F., Wang, N., Shen, X., & Yang, S. (2022). Dietary carotenoid intake and osteoporosis: The national health and nutrition examination survey (2005–2018). *Arch. Osteoporos., 17*, 1–8. https://doi.org/10.1007/s11657-021-01047-9

Kassim, A., Workneh, T. S., & Bezuidenhout, C. N. (2013). A review on postharvest handling of avocado fruit. *African Journal of Agricultural Research, 8*, 2385–2402. https://doi.org/10.5897/AJAR12.1248

Kruk, J., Szymanska, R., Cela, J., & Munné-Bosch, S. (2014). Plastochromanol-8: Fifty years of research. *Phytochemistry, 108*, 9–16. https://doi.org/10.1016/j.phytochem.2014.09.011

Lichtenthaler, H. K., & Buschmann, C. (2001). Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. *Curr. Protoc.Food Anal. Chem., F4*(3), 1–8.

Muñoz, P., & Munné-Bosch, S. (2018). Photo-oxidative stress during leaf, flower and fruit development. *Plant Physiology, 176*, 1004–1014. https://doi.org/10.1104/pp.17.01127

Olivares, D., Alvarez, E., Véliz, D., García-Rojas, M., Díaz, C., & Defilippi, B. G. (2020). Effects of 1-methylcyclopropene and controlled atmosphere on ethylene synthesis and quality attributes of avocado cvs. *Edranol and Fuerte. J. Food Qual.*. , Article 5075218. https://doi.org/10.1155/2020/5075218

Pérez, K., Mercado, J., & Soto-Valdez, H. (2004). Effect of storage temperature on the shelf life of hass avocado (*Persea americana*). *Food Science and Technology International, 10*, 73–77. https://doi.org/10.1177/1082013204043763

Power, R., Nolan, J. M., Prado-Cabrero, A., Roche, W., Coen, R., Power, T., & Mulcahy, R. (2022). Omega-3 fatty acid, carotenoid and vitamin E supplementation improves working memory in older adults: A randomised clinical trial. *Clinical Nutrition, 41*, 405–414. https://doi.org/10.1016/j.clnu.2021.12.004

Prabath Pathirana, U. A., Sekozawa, Y., Sugaya, S., & Gemma, H. (2013). Changes in lipid oxidation stability and antioxidant properties of avocado in response to 1-MCP and low oxygen treatment under low-temperature storage. *Int. Food Res. J., 20*, 1065–1075.

Ramel, F., Birtic, S., Cuiné, S., Triantaphylidès, C., Ravanat, J. L., & Havaux, M. (2012). Chemical quenching of singlet oxygen by carotenoids in plants. *Plant Physiology, 158*, 1267–1278. https://doi.org/10.1104/pp.111.182394

Shahidi, F. (2015). *Handbook of antioxidants for food preservation*. Kidlington, U.K: Elsevier, 978-1-78242-7.

Shewfelt, R. L., & del Rosario, B. A. (2000). The role of lipid peroxidation in storage disorders of fresh fruit and vegetables. *HortScience, 35*, 575–579. https://doi.org/10.21273/HORTSCI.35.4.575

Vincent, C., Mesa, T., & Munné-Bosch, S. (2020a). Identification of a new variety of avocados (*Persea americana* Mill. cv. Bacon) with high vitamin E and impact of cold storage on tocochromanols composition. *Antioxidants, 9*, 403. https://doi.org/10.3390/antiox9050403

Vincent, C., Mesa, T., & Munné-Bosch, S. (2020b). Hormonal interplay in the regulation of fruit ripening and cold acclimation in avocados. *Journal of Plant Physiology, 251*, Article 153225. https://doi.org/10.1016/j.jplph.2020.153225

Vincent, C., & Munné-Bosch, S. (2022). Quality determination of avocado fruit immersed in a pyridoxal 5'-phosphate solution. *Journal of Food Composition and Analysis, 110*, Article 104526. https://doi.org/10.1016/j.jfca.2022.104526

Wang, W., Bostic, T. R., & Gu, L. (2010). Antioxidant capacities, procyanidins and pigments in avocados of diferent strains ad cultivars. *Food Chemistry, 122*, 1193–1198. https://doi.org/10.1016/j.foodchem.2010.03.114

Weber, P., Bendich, A., & Machlin, L. J. (1997). Vitamin E and human health: Rationale for determining recommended intake levels. *Nutrition, 13*, 450–460.

Yahia, E. M., & Woolf, A. B. (2011). Avocado (*Persea americana* Mill.). In A, & E. M. Yahia (Eds.), *Postharvest biology and technology of tropical and subtropical fruit* (pp. 125–186). Woodhead Publishing Series in Food Science, Technology and Nutrition, ISBN 9781845697341. https://doi.org/10.1533/9780857092762.125.

Zauberman, G., & Jobin-Decor, M. P. (1995). Avocado (Persea americna Mill.) quality changes in response to low-temperature storage. *Postharvest Biology and Technology, 5*, 235–243. https://doi.org/10.1016/0925-5214(94)00027-P

Zhang, Y., Butelli, E., De Stefano, R., Schoonbeek, H., Magusin, A., Pagliarani, C., Wellner, N., Hill, L., Orzaez, D., Granell, A., Jones, J. D. G., & Martin, C. (2013). Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold. *Current Biology, 23*, 1094–1100. https://doi.org/10.1016/j.cub.2013.04.072