Maintaining postharvest quality of cold stored 'Hass' avocados by altering the fatty acids content and composition with the use of natural volatile compounds – methyl jasmonate and methyl salicylate

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Abstract

BACKGROUND: Low temperature is often used to reduce metabolic processes and extend the storage of fruit, however, in the case of avocado the temperature below 3 °C would often result in development of physiological disorders associated with chilling injury. The objective of this study was to investigate the ability of methyl jasmonate (MeJA) and methyl salicylate (MeSA) vapours to alleviate the chilling injury in 'Hass' avocado fruit kept at 2 °C for 21 d followed by 6-7 d shelf-life at 20 °C, simulating supply chain conditions.

RESULTS: The incidence and severity of chilling injury was significantly reduced in MeJA and MeSA exposed fruit, especially at 100 µmol l^{-1}. The mechanism involved improved membrane integrity via alteration of the fatty acids content and composition, down-regulation of LOX gene expression and reduced activity of lipoxygenase.

CONCLUSION: Methyl jasmonate and methyl salicylate have the potential for being used with 'Hass' avocado fruit shipped at low temperature by reducing their susceptibility to chilling injury.

Keywords: *Persea americana* Mill., chilling injury, fatty acids, methyl jasmonate, methyl salicylate, supply chain

INTRODUCTION

The avocado fruit (*Persea americana* Mill.) is becoming more and more popular among the consumers, especially due to being rich in nutrients associated with health benefits.[1] The fruit also contains high level of fatty acids, i.e. oleic (a monounsaturated omega-9 fatty acid, C18:1) and palmitic (a saturated fatty acid, C16:0) acid,[2-4] while the sugar content is relatively low.

According to the latest (2015) food trade and supply chain directory (www.foodtradesa.co.za), the European Union, and United Kingdom in particular, is the biggest export market for the South African avocados.

The fruit are transported by the sea freight, which means that it may take up to four weeks from harvest before reaching the consumer.[5] The standard shipping temperature used by the South African avocado industry for 'Hass' avocados is around 5.5 °C; however, to prevent 'soft landing' at the destination port, industry often uses an ethylene inhibitor 1-methylcyclopropene (1-MCP) during the shipment under controlled atmosphere conditions (2-5% O_2, 5-10% CO_2).[6,7] Unfortunately, the application of 1-MCP causes problems, especially with the 'Hass' avocado fruit destined for the 'ready to eat' ripening programmes, due to uneven ripening (firmness) and poor fruit colouration.[8] The fact that good appearance and the right firmness of the produce have huge impact on consumers' willingness to buy the fruit has been highlighted by several authors.[9,10] Furthermore, the delayed ripening caused by 1-MCP aggravates the incidence of postharvest fungal rots.[11]

The South African avocado industry is also planning to expand into the new markets, e.g. US. This would require disinfestation treatment before the fruit could enter the country. The exposure to temperatures ranging from 1.1 to 2.2 °C for 14–18 d has been proven to control fruit flies and thus could be used as a cold quarantine treatment.[12] Low temperatures are often used to reduce metabolic processes and extend storage life of the fruit, however, during the extended period of cold storage at temperature less than 3 °C avocado fruit are likely to develop physiological disorders associated with chilling injury,[13-15] which may be expressed in several ways, such as skin blackening, pitting or sunken lesions, lenticels damage, and mesocarp discolouration, e.g. internal browning and/or grey pulp. The main problem with the internal disorders in the ripe fruit is the fact that it is not possible to notice them from the outside. Thus, it is not surprising that the lack of internal defects has been identified[16] among the key aspects of avocado quality that ensure consumers' satisfaction.

Internal browning is related to textural quality loss due to alterations in membranes permeability, allowing polyphenol oxidase (PPO) to come into contact with its substrates - phenolic compounds. PPO catalyses the oxidation of phenols into quinones that are subsequently polymerised into brown pigments.[6,17]

The postharvest use of jasmonates and salicylates seems to have unexplored potential.[18] Their ability to alleviate chilling injury has been demonstrated in numerous tropical and sub-tropical fruit, i.e. in guava,[19] loquat,[20,21] mango,[22,23] papaya,[24] peach,[25,26] and pomegranate.[27] It has also been reported that dipping 'Hass' avocado fruit in 2.5 µmol l^{-1} methyl jasmonate solution for 30 s reduced the development of chilling injury in fruit subsequently stored for 2 weeks at 1 °C[28] and 4 weeks at 2 °C,[29] respectively. To the best of our knowledge, there are only two reports[28,29] on the use of methyl jasmonate with avocado fruit mainly focused on dipping treatment, with very little information on the vapour treatment.[29] There is also an absolute lack of information in the literature on the effects of salicylates on avocado fruit quality during low temperature storage.

Thus, the objective of this study was to investigate the effect of methyl jasmonate (MeJA) and methyl salicylate (MeSA) vapours exposure at two concentrations (10 and 100 µmol l^{-1}) on i) chilling injury incidence and severity, ii) flesh colour, iii) activity of L-phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and lipoxygenase (LOX) enzymes iv) expression of PAL and LOX genes and v) fruit composition in terms of its phytonutrient content (fatty acids, phenolic compounds, and D-mannoheptulose) in 'Hass' avocado fruit kept at 2 °C for 21 d followed by 6-7 d shelf-life at 20 °C, simulating quarantine treatment and supply chain conditions.

EXPERIMENTAL

Plant material and handling

Freshly harvested, unblemished 'Hass' avocado fruit were obtained from Koeltehof Packers (Nelspruit, Mpumalanga province, South Africa) at commercial maturity. Fruit were transported to the laboratory and then i) left untreated - control ii) dipped for 5 min in 0.05 % prochloraz® - the commercial treatment; iii) exposed to methyl jasmonate (MeJA) or methyl salicylate (MeSA) vapours at two concentrations of 10 and 100 µmol l^{-1} for 24 h at 20.0±0.5 °C.

After placing the fruit in a 10 l air-tight sealable plastic containers container, the appropriate volume of MeJA or MeSA to reach the desired concentration of 10 and 100 µmol l^{-1}, respectively was spotted on the Petri dish at the bottom of the container, as previously described for thyme oil fumigation.[30] The container was immediately hermetically-sealed and solutions were left to evaporate over the 24 h period. Control and prochloraz treated fruit were also kept in similar sealed containers. Avocado fruit were subsequently stored for 21 d at 2.0±0.2 °C, respectively to simulate the shipping conditions, and thereafter kept at 20.0±0.5 °C, RH 70 % for 6-7 d to simulate the retail shelf-life conditions. Each treatment had six replicate boxes, each containing eighteen fruit, i.e. 108 fruit per treatment, equalling to a total of 648 fruit per trial. The experiment was repeated twice.

Evaluation of chilling injury incidence and severity

The incidence of chilling injury was recorded as a % of fruit with signs of mesocarp discolouration due to cold damage. The severity of the chilling injury was rated according to the chilling index (Fig. 1) on a scale from 0 to 5, at the 'ripe and ready to eat' stage, i.e. after 21 d of cold storage plus 6-7 d shelf-life at 20 °C.

Disease incidence

At the 'ripe and ready to eat' stage fruit were assessed for signs of rotting (anthracnose), by giving them a score of 0 or 1 – no/ signs of rotting, respectively. Disease incidence was expressed as the proportion (%) of fruit showing signs of rotting out of the total number of fruit in each treatment.

Physical properties of the fruit

Flesh firmness was determined along the equator of the fruit using a Chatillon Penetrometer, Model DFM50 (Ametek, Largo, Florida, USA) with an 8 mm diameter flat-head stainless steel cylindrical probe - puncture test.[31] Flesh colour measurements were taken at three points from each half of the fruit with a Minolta Chroma meter CR 0-2000 (Minolta Camera Co. Ltd, Tokyo, Japan), calibrated with manufacturer's standard white plate.[32]

Biochemical analyses

Determination of PAL activity was conducted following the method previously described in Sellamuthu *et al.*[33], with some modifications. Fruit tissue sample (1.0 g) was homogenised with borate buffer (100 mmol l^{-1}, pH 8.8) containing 5 mmol l^{-1} of *β*-mercaptoethanol and 2 mmol l^{-1} EDTA. 75 µl of the extract was then incubated for 60 min at 37 °C with 150 µl of borate buffer (50 mmol l^{-1}, pH 8.8) containing 20 mmol l^{-1} L-phenylalanine. After incubation time, the reaction was stopped by adding 75 µl of 1 mol l^{-1} HCl and the production of cinnamate was spectrophotometrically determined at 290 nm (Zenyth 200rt Microplate Reader UK-Biochrom Ltd.). The specific activity of enzyme was expressed as nmol cinnamic acid h^{-1} mg^{-1} of protein.

Phenolic acids (*p*-coumaric acid, ferulic acid, caffeic acid, protocatechuic acid, 4-hydroxybenzoic acid, and pyrogallol) content in the fruit mesocarp was determined according to the method of Zhang and Zuo[34] with some modifications. Freeze-dried samples (5 g) were extracted with 40 ml of 70 % (v/v) methanol (MeOH). After extraction 100 µl of naphthol (1 µmol l^{-1}) were added as an internal standard. The mixture was agitated and subsequently incubated for 2 h at 60 °C. The samples were centrifuged and the resulting 500 µl of supernatants were dried in a SpeedVac™ (Thermo Fisher Scientific, Massachusetts, US). Dried samples were reconstituted with 150 µl of acetonitrile, followed by 50 µl BSTFA [N,O-bis (trimethylsilyl) trifluoroacetamide)] and 1 % TMCS (trimethylchlorosilane). BSTFA and TMCS were used as silylation reagents. The mixture was agitated and then derivatised by incubating for 1 h at 80 °C. After incubation, the mixture was agitated again and then transferred into GC vials. One µl was injected into a GC/MS (7890A Gas Chromatograph with 5975C Mass Spectrometer; Agilent Chemetrix (Pty) Ltd,

Johannesburg, South Africa). Phenolic acids separation was performed on a DB-5ms Ultra Inert, 30 m x 0.25 mm, 0.25 µm column. The GC/MS conditions and run parameters were set according to Zhang and Zuo[34]. Helium was used as a carrier gas at flow rate of 1 ml min^{-1}. Identification of phenolic compounds was achieved by matching the peaks retention times (RT) with authentic standards. The individual compounds were quantified by calculating their peak areas, and their content was expressed on dry weight (DW) basis.

The polyphenol oxidase (PPO) activity was determined using a method described by Soliva *et al.*[35] with minor modifications. The 20 µl of enzyme and 200 µl buffered substrate (0.1 mol l^{-1} sodium phosphate, pH 7.0 and 0.05 mol l^{-1} catechol) were mixed thoroughly. Afterwards, the increase in absorbance at 410 nm was measured for 5 min (SPECTROstarNano Microplate Reader, BMG LABTECH, Ortenberg, Germany). The specific activity of the enzyme was expressed as µmol mg^{-1} of protein.

For the determination of fatty acids, i.e. oleic acid (C18:1, a monounsaturated omega-9 fatty acid), palmitic acid (C16:0, a saturated fatty acid), palmitoleic acid (C16:1, a monounsaturated omega-7 fatty acid), and linoleic acid (18:2, a polyunsaturated omega-6 fatty acid) 1 g of lyophilised mesocarp tissue powder was homogenised with 30 ml of hexane for 30 s. Oil extraction was performed following the method used by Meyer and Terry[36]. Avocado oil extract was dissolved in 2 ml of hexane and mixed with 0.2 ml of 0.2 mol l^{-1} potassium hydroxide (KOH) in methanol. The mixture was shaken vigorously for 30 s, and left until the upper hexane layer became clear. This layer containing methyl esters was decanted and diluted 1:100 (v/v) with fresh hexane directly prior to the injection into the GC/MS. Fatty acids separation was performed on a HP-88 112-8867, 60 m x 0.25 mm, 0.20 µm column using helium as a carrier gas at constant flow rate of 1.4 ml min^{-1}. The GC conditions and run parameters were set up according to Meyer and Terry[36]. Fatty acids were identified and quantified using known concentrations of the standards, i.e. methyl palmitate, methyl oleate and methyl linoleate. Fatty acids content was expressed on dry weight (DW) basis.

LOX activity was determined following the method used by Maalekuu *et al.*[37]. Briefly, avocado pulp tissue (15 g) was homogenized in 30 ml of phosphate buffer (0.05 mol l^{-1}, pH 7.0) for 1 min using a Janke & Kunkel IKA Labortechnik ultra-turrax T25 homogenizer (IKA, Germany). The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C and filtered. Turbidity was cleared with a few drops of 2 N NaOH. The crude extract was mixed with the substrate – linoleic acid (20 µl) in a spectrophotometer cuvette containing 3 ml of 0.2 mol l^{-1} phosphate buffer, pH 6.5 and the absorbance was measured at 234 nm at 6 s intervals for 1 min using spectrophotometer (SPECTROstarNano Microplate Reader, BMG LABTECH,

Ortenberg, Germany). The rate of formation of conjugated diene reaction products was measured as an increase in absorbance at 234 nm, and specific enzyme activity was calculated and expressed as U mg^{-1} protein h^{-1}. Protein concentration was determined by the method of Bradford[38].

Mannoheptulose was extracted following the method of Roessner *et al.*[39] with some modifications. Freeze-dried powder (100 mg) was dissolved in 1.4 ml of 100 % methanol and 50 µl of internal standard (2 g l^{-1} ribitol (w/v) in water) was added. The mixture was agitated for 10 s and subsequently extracted for 15 min at 70 °C. Samples were then centrifuged at 6000 x *g* for 10 min, and supernatants were transferred to the new tubes, where 750 µl of dichloromethane and 1.5 ml of distilled water were added. The tubes were agitated for 15 s and centrifuged again at 6000 x *g* for 15 min. Thereafter, 150 µl of the upper (polar) phase were transferred into the new tubes and evaporated under nitrogen gas until reaching complete desiccation. For identification and quantification, the residue was re-suspended and derivatised for 2 h at 37 °C in 40 µl of 20 g l^{-1} methoxyamine hydrochloride in pyridine, with occasional shaking. This was followed by a 30 min incubation with 70 µl N-methyl-N-(trimethylsilyl) trifluoroacetamide at 37 °C. After derivatisation, the samples were transferred into injection vials. Derivatised sugars were analysed using GC/MS with an Agilent J&W DB-17 (50 %-phenyl)-methyl-polysil-oxane column 30 m x 250 µm x 0.25 µm, with helium as a carrier gas at flow rate of 1 ml min^{-1}. The GC conditions and run parameters were set up according to Roessner *et al.*[39]. D-mannoheptulose sugar was identified and quantified by comparison of peak area with that of known standard, i.e. - D-mannoheptulose (≥ 99.0%, Sigma Aldrich). Mannoheptulose content was expressed on dry weight (DW) basis.

Gene expression analysis

The analysis of expression of PAL and LOX genes was performed by qPCR, using a SYBR-green dye system, as described in Bill.[40] The comparative $-\Delta\Delta C_t$ method was employed to evaluate the relative quantities of each of the amplified products. RNA Extraction: High quality total RNA was obtained from the fruit by using the total RNA plant mini kit (Zymo Research Corporation, Inqaba Biotech, South Africa). The RNA samples were analysed by electrophoresis in 1.8 % (w/v) agarose and formaldehyde denaturing gel. The purity of RNA purity was assessed using a BioPhotometer plus (Eppendorf Inc., Westbury, NY, USA). Reverse Transcription: A total of 1µg RNA was used for cDNA synthesis with RT-PCR, using transcriptor first strand cDNA synthesis kits (Roche Applied Science, Penzberg, Upper Bavaria, Germany). Primers and Reference Gene Selection: Specific primer sets were designed using the Primer3 software (http://biotools.umassmededu/bioapps/primer3_www.cgi) from the specific sequence of *Persea americana*

deposited in the NCBI GenBank (Supplementary material). The primer pairs were chosen and validated *in silico* using primer BLAST specific analysis (http://www.ncbi.nlm.nih.gov/Blast.cgi) and then according to the melting profiles obtained from the quantitative real time PCR conditions (qPCR). To determine the specificity of the amplicons, melting curve analysis was performed over the range of temperatures from 60 to 95 °C. Quantitative RT-PCR: The qPCR was performed in UltraFlux® 96-well PCR Plate (SSI, Lodi, CA, USA) using the PowerUP™ Sybr® Green Master mix (Applied Biosystems, Austin, TX, USA) on a CFX Connect™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) under the following conditions: an initial holding cycle (10 min at 95 °C), followed by 40 cycles of two steps of denaturation and annealing/extension (15 s at 95 °C and 60 s at 60 °C respectively). The PCR amplification was carried out in a total volume of 20 µl, containing 1 µl of diluted (1:20) cDNA (5 ng μl^{-1}), 0.25 µmol l^{-1} of each primer, and 10 µl of Sybr® Green Master mix. The qPCR efficiency for each set of primers was determined using standard curves generated with five cDNA pool dilutions, i.e. undiluted, and at following dilutions: 1:4, 1:16, 1:64, and 1:256, respectively.

Statistical analyses

Avocado fruit were organised in 6 replicates of 18 fruit for each of the six treatments. Data are presented as mean values from a fully randomised design. Data were subjected to analysis of variance (ANOVA) to identify the treatments that had significant effect on the characteristics of 'Hass' avocado fruit, as determined with Fisher's Least Significant Difference (LSD) test with a significance level of 0.05, using GenStat 18th Edition software.

RESULTS AND DISCUSSION

Chilling injury incidence and severity

'Hass' avocado fruit are susceptible to cold damage if kept at the temperature below 3 °C,[13-15] thus it is not surprising that the majority (around 80 %) of the untreated fruit developed mesocarp disorders when held at 2 °C for 21 d followed by 6-7 d shelf-life at 20 °C (Fig. 2A). Chilling injury incidence was also high (above 60 %) in fruit treated with prochloraz. On the other hand, incidence of chilling injury was substantially reduced to around 20 % in fruit treated with MeJA or MeSA at 100 µmol l^{-1}. A reduction in chilling injury was a bit less pronounced at the lower concentration of 10 µmol l^{-1}. The severity of chilling injury (chilling index) across the batches of fruit was significantly reduced in fruit exposed to MeJA at 10 µmol l^{-1} (1.6) or 100 µmol l^{-1} (1.3) and MeSA at 10 µmol l^{-1} (1.0) or 100 µmol l^{-1} (1.1) when compared with untreated fruit (2.6) and those treated with prochloraz (2.2).

Reduced chilling injury in avocado fruit exposed to MeJA and MeSA is in agreement with majority of studies reviewed by Glowacz and Rees[18] where numerous tropical and sub-tropical fruit were exposed to MeJA or MeSA prior to storage, and also with those where 'Hass' avocado fruit were dipped in 2.5 µmol l^{-1} MeJA solution for 30 s prior to storage.[28,29]

Disease incidence

Disease incidence was observed in around 60 % of the untreated fruit, whereas it was significantly (P<0.05) reduced in fruit treated with prochloraz (26.9 %), MeJA at 10 µmol l^{-1} (27.5 %) and 100 µmol l^{-1} (22.4 %), or MeSA at 10 µmol l^{-1} (17.9 %) and 100 µmol l^{-1} (16.4 %). This observation is not surprising since chilling injured fruit are often more susceptible to fungal infections.[41] A slightly lower disease incidence in fruit exposed to MeSA could be associated with its ability to reduce the ethylene production.[42]

Physical properties of the fruit

All fruit were assessed at the 'ripe and ready to eat' stage - at firmness being in the range from 0.65 to 0.90 kg, i.e. below 1 kg, which according to the International Organization for Standards (ISO 7619) represents ripe fruit.

The storage of avocado fruit at low temperature may cause lenticels damage, however in the case of 'Hass' avocados exported to the UK, which are marketed as a 'ripe and ready to eat' fruit, this may not be a serious issue due to the fact that lenticels damage can only be noticed on green unripe fruit, while the product would be displayed on the market shelf when ripe (purple/black), so the external chilling injury would easily be masked.[43] For this reason, and also because as mentioned before, the lack of internal defects being among the key aspects of avocado fruit quality that ensures consumers' satisfaction,[16] this research focused on the internal appearance of the fruit. In terms of colour, the mesocarp of all the fruit treated with either MeJA or MeSA was lighter (higher L^* value), greener (lower a^* value) and more yellow (higher b^* value) than untreated control (Table 1), further confirmed by the higher *hue*° value, while in comparison with the fruit treated with prochloraz the differences were less pronounced. This could be related to the lower incidence and severity of mesocarp browning caused by chilling injury (Fig. 2A) or postharvest decay in those fruit when compared with the severely affected untreated control. Reduced mesocarp browning is in agreement with previously reported studies, where loquat fruit were exposed to MeJA at 10 µmol l^{-1} for 24 h,[44] and peaches were exposed to MeJA at 1 µmol l^{-1} for 24 h[45,46] or 16 µmol l^{-1} for 6 h,[47] before being subsequently stored at 0–1 °C.

Fruit biochemistry

Phenolic acids, activity of PAL and PPO

Mesocarp browning is one of the main causes of quality loss,[16] especially during the storage at low temperature.[14,15] The key components involved in this process are phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) involved in the biosynthesis of phenolic compounds, phenolics content, polyphenol oxidase (PPO; EC 1.14.18.1) activity, and loss of tissue integrity[48,49] because the substrates and enzymes are separated from each other by being in a different cellular compartments, thus tissue browning does not progress until the membrane integrity is reduced, and they come into contact.

The activity of PAL (Fig. 3) and also PAL gene expression (Fig. 4) were increased in fruit treated with prochloraz, and MeJA or MeSA at 100 µmol l^{-1} but not in those treated at 10 µmol l^{-1}, which would suggest that phenolics biosynthesis would be higher in these samples. Phenolic compounds improve the antioxidant capacity and reactive oxygen species (ROS) scavenging activity, thus they could also contribute to reduced susceptibility to chilling damage.[44]

The exposure to both MeJA and MeSA affected the composition of individual phenolic compounds in the avocado fruit. Exposure to MeJA at 100 µmol l^{-1} resulted in significantly higher content of pyrogallol, caffeic acid, and hydroxybenzoic acid, while the content of protocatechuic acid was lower when compared with untreated fruit and those treated with prochloraz prior to storage (Table 2). Similar effect was observed in fruit exposed to MeSA at 10 µmol l^{-1} and 100 µmol l^{-1} but not in those exposed to MeJA at 10 µmol l^{-1} (Table 2), where only the pyrogallol content was higher while other phenolics were not affected. The lowest content of pyrogallol observed in control and prochloraz treated fruit could be to some extent explained by pyrogallol being used as a substrate by polyphenol oxidase (PPO) and peroxidase (POD) leading to tissue browning as indicated by higher chilling index,[17,50] especially taking into account high affinity of PPO for pyrogallol. The content of *p*-coumaric acid and ferrulic acid, which usually increase during the fruit ripening,[51] was not affected by the treatments, suggesting that fruit were likely at the same ripeness stage.

On the other hand the activity of PPO was highest in the control fruit, while in all other treatments, with an exception of MeJA at 10 µmol l^{-1} that had no effect, the activity of PPO was significantly reduced (Fig. 5). The ability of salicylate to reduce the activity of PPO and POD has recently been reported.[52] Highest activity of PPO, assuming reduced tissue integrity being in place,[53] would definitely be responsible for the severe mesocarp browning in untreated fruit.[6]

Care needs to be taken when directly comparing the activity of these enzymes among the treatments, because as indicated by chilling injury incidence and severity, the tissue integrity was not the same. Thus, even though the higher activity of PPO led to severe tissue browning in untreated fruit, this might not necessary be the case in the fruit exposed to MeJA at 10 μmol l^{-1}; chilling injury incidence was indeed higher in these samples when compared to their counterparts exposed to MeJA at 100 μmol l^{-1} and MeSA at 10 μmol l^{-1} and 100 μmol l^{-1}, but much lower than in case of untreated fruit and those treated with prochloraz (Fig. 2A). Improved membrane integrity has in fact been reported for numerous produce treated with jasmonates and salicylates prior to cold storage, e.g. in loquat treated with MeJA at 10 μmol l^{-1} for 24 h, stored at 1 °C,[20] pomegranates treated with MeJA and MeSA at 10 and 100 μmol l^{-1} for 16 h, stored at 2 °C,[27] and tomatoes treated with MeSA at 50 μmol l^{-1} for 12 h, stored at 2 °C.[54]

Fatty acids

Total fatty acids content was found to be significantly reduced in untreated (102.56 g kg^{-1}) and prochloraz (105.53 g kg^{-1}) treated fruit, compared with those treated with MeJA at 10 μmol l^{-1} (121.49 g kg^{-1}) and 100 μmol l^{-1} (122.56 g kg^{-1}), and also those exposed to MeSA at 10 μmol l^{-1} (118.85 g kg^{-1}) and 100 μmol l^{-1} (124.03 g kg^{-1}), respectively. The lower fatty acids content in untreated and prochloraz treated fruit could be a result of lipid degradation[53,55] due to excess ROS production.

The main fatty acid identified in avocado fruit was oleic acid (18:1). The higher proportion of monounsaturated fatty acids is in agreement with the published literature.[56-58] There was a clear effect of MeJA and MeSA exposure on individual fatty acid composition (Table 3), where oleic acid content was increased. In case of the other fatty acids MeJA and MeSA affected them in a different way. In 'Hass' avocado fruit exposed to MeJA the content of palmitic acid (16:0) was significantly (P<0.05) lower, while linolenic acid (18:2) was slightly but not significantly higher when compared with untreated fruit and those treated with MeSA. This led to unsaturated: saturated fatty acids ratio being significantly higher in MeJA exposed fruit compared with all the other treatments. Similar observation has recently been reported in olives exposed to MeJA prior to storage.[59] On the other hand, in avocado fruit exposed to MeSA, only oleic acid (18:1) content was significantly (P<0.05) increased compared to untreated and prochloraz treated fruit, whereas linoleic acid (18:2) was not affected, which led to the highest monounsaturated: polyunsaturated ratio in those samples, especially where MeSA was applied at 100 μmol l^{-1}. These changes would surely affect the stability of membranes, since at low temperature membranes need to have higher content of unsaturated fatty acids to maintain their functionality.[60,61]

It is well known that high LOX activity would lead to reduced membrane integrity during the chilling stress. LOX gene expression was significantly down-regulated in all the fruit treated with either MeJA or MeSA, especially at 100 µmol l^{-1} (Fig. 6); similar trend was observed for the LOX activity. These results are in agreement with previously reported findings from avocados dipped in 2.5 µmol l^{-1} MeJA solution for 30 s and subsequently stored at 1 °C[28] and loquat exposed to 10 µmol l^{-1} MeJA for 24 h prior to storage at 1 °C,[44] and highlight the ability of these treatments to alter membrane stability.

D-mannoheptulose, (C7 sugar), is known to be the main sugar in avocado fruit,[36,62] whereas fructose and glucose that are often found in majority of fruit, have been reported to be only present at very low concentrations in avocados.[36] There was no significant difference in the content of D-mannoheptulose between untreated control, and either prochloraz treated fruit or those exposed to MeSA at 10 µmol l^{-1} and 100 µmol l^{-1}. However, significantly (P<0.05) higher content of D-mannoheptulose was observed in fruit exposed to MeJA at 100 µmol l^{-1} (Table 4), while at 10 µmol l^{-1} the content of D-mannoheptulose was only slightly and not significantly higher than in control fruit. This could be associated with differences in the membrane stability as fruit biochemistry (PAL, phenolics and fatty acid composition) and chilling index were not exactly the same for fruit treated with MeJA at 10 µmol l^{-1} and 100 µmol l^{-1}.

It has been previously suggested that higher content of D-mannoheptulose is responsible for delayed ripening since higher content of this sugar was reported for unripe and ripening fruit compared with the 'ripe and ready to eat' fruit.[36,56,63,64] This finding was further confirmed in fruit exposed to 1-MCP, where ripening was inhibited/delayed and D-mannoheptulose was maintained at higher level.[36] Thus, even though in this research the D-mannoheptulose content was found to be significantly higher in one of the treatments (MeJA at 100 µmol l^{-1}) it is unlikely that the fruit were at different stage of ripeness, based on the physical properties, i.e. firmness and flesh colour (Table 1) of the fruit were not that different among the treatments. Furthermore, Pedreschi *et al.*[4] has recently found no direct correlation between the content of D-mannoheptulose and time to reach edible ripeness in the 'Hass' avocado fruit.

CONCLUSIONS

Exposure of 'Hass' avocado fruit to methyl jasmonate (MeJA) and methyl salicylate (MeSA) vapours, especially at 100 µmol l^{-1} for 24 h prior to storage at 2 °C reduced their susceptibility to chilling injury. The findings from this research highlight the potential of these treatments for being used if 'Hass' avocado fruit are to be shipped at low temperature. Their mode of action is based on the ability of these treatments to alter membrane integrity and fatty acids content/composition via down-regulating the activity of LOX.

Future studies, however should focus on studying, in more depth, the effects of MeJA and MeSA exposure on the functionality of cell membranes in avocado fruit stored at low temperature, i.e. 1-2 °C to better understand the exact mechanism of action for these signalling compounds, as membranes are the primary sites for development of chilling injury.

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