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The physiological effect of fruit maturity and 1-methylcyclopropene on 'Hass' avocado fruit exocarp colour and chilling injury during ripening

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ABSTRACT

Purpose: This study was undertaken to investigate the influence of harvest maturity and 1-methylcyclopropene (1-MCP) treatment on exocarp colour development and chilling injury of 'Hass' avocado fruit during ripening. **Research method:** 'Hass' avocado fruit harvested at three different maturity stages, early (21% DM), mid- (28% DM) and late (35% DM) were treated with 300 g mol^{-1} of 1-MCP for 16 hours and stored at 5.5 °C for 28 days, subsequently, ripened at 21 °C. The physico-chemicals quality parameters evaluated every 2 days includes exocarp subjective (visual colour), objective (lightness-L^*, chroma-C^* and hue angle-h^o), chlorophyll-*a* and -*b*, total anthocyanin, cyanidin 3-*O*-glucoside and chilling injury. **Findings:** 1-MCP delayed ripening for early and mid-matured fruit and extended ripening by 2-4 days when compared with untreated fruit. In this study, exocarp colour development of 'Hass' avocado fruit was improved by 1-MCP treatment as measured visual and objectively. The accumulation of total anthocyanins and cyanidin 3-*O*-glucosides in 1-MCP-treated fruit was slower than that in untreated fruit, however, 1-MCP treatment was associated with higher concentrations after fruit had reached 'eat-ripe' firmness, irrespective of maturity. The study found that 1-MCP reduced the development of chilling injury symptoms for early harvested and mid-harvested 'Hass' avocado fruit. **Research limitations:** The main limitation of the present study is the lack of evaluation of ethylene production. **Originality/Value:** The study found that different maturity stages of 'Hass' avocado fruit responded differently to 1-MCP treatment. Thus, 1-MCP had a positive effect on early and mid-harvest fruit exocarp colour and CI development during ripening.

INTRODUCTION

Avocado (*Persea americana* Mill.) 'Hass' is a member of the Lauraceae family (Chanderbali et al., 2008) with spherical-shaped and medium size fruit that pleasant, creamy and smooth texture edible covered by the thick dark green exocarp (Hurtado-Fernández et al., 2016). Its unique exocarp colour development from green to purple then black during ripening has made 'Hass' very popular among avocado cultivars (Cox et al., 2004). The exocarp colour is used as a visual quality parameter for assessing postharvest fruit quality and marketability of 'Hass' avocado fruit. The importance of this quality attribute in limiting marketability and consumer satisfaction has been repeatedly studied (Mathaba et al., 2015; Mathaba et al., 2017). Consumers associate purple colour development with ripeness and good quality thus, driving preference and purchase at retail stores. Colour development for 'Hass' avocado fruit can be strongly affected by harvest maturity and postharvest treatments used during storage (Mathe et al., 2018).

In avocado fruit, harvest maturity is important for quality and handling and markedly influences colour development during ripening. Therefore, harvesting 'Hass' avocado fruit at an early maturity stage results in poor colour development during ripening and as such fruit quality becomes insufficient to fulfil traders and consumer preference (Mathaba et al., 2015). The purple and black exocarp colours are mainly determined by anthocyanin pigments identified as cyanidin 3-*O*-glucoside (Cox et al., 2004). Previous studies showed that the concentration of cyanidin 3-*O*-glucoside, which contributes to 'Hass' avocado fruit purple exocarp colouration, was significantly influenced by harvest maturity (Ashton et al., 2006; Cox et al., 2004). Donetti & Terry (2014) found that 'Hass' avocado fruit harvested at early maturity showed reduced exocarp cyanidin 3-*O*-glucoside concentration during ripening. However, fruit harvested at late maturity recorded higher cyanidin 3-*O*-glucoside concentrations accompanied by improved colour change (Cox et al., 2004).

Postharvest treatment such as 1-methylcyclopropene (1-MCP) has been used to extend the storage-life of avocado fruit during cold storage (Jeong et al., 2002). In general, 1-MCP application inhibits ethylene perception, consequently altering the ethylene-dependent process including softening and colour development (Hershkovitz et al., 2005; Jeong et al., 2002; Mubarok et al., 2022). Moreover, the influence of 1-MCP on fruit quality attributes such as softening, and colour development is by delaying their metabolic rate. Several studies showed that 1-MCP treatment for avocado fruit resulted in delayed softening, colour development and incidence of physiological disorders (Woolf et al., 2005). Research findings have revealed that the effectiveness of 1-MCP was ascribed to harvest maturity (Blankenship & Dole, 2003; Satekge & Magwaza, 2022). Moreover, avocado fruit are sensitive to low temperatures, and prolonged storage under 10 °C causes chilling symptoms such as darkening of the surface, pitting, discolouration of the mesocarp, uneven ripening, and poor fruit quality (Setagane et al., 2021). the development of chilling injury symptoms for 'Hass' avocado fruit exacerbates the desynchronization of fruit firmness with exocarp colour change during ripening. During ripening, Mathaba et al. (2015) found that the development of CI symptoms was closely related to poor exocarp colour changes for 'Hass' avocado fruit. According to studies, 1-MCP can increase or decrease chilling injury, and has been widely used to reduce postharvest chilling injury in climacteric fruit crops. However, little has been documented about its relationship with anthocyanin accumulation subsequently leading to exocarp colour development in 'Hass' avocado fruit during ripening. The objective of this study was to investigate the influence of harvest maturity and 1-MCP treatment on exocarp colour development and chilling injury of 'Hass' avocado fruit during ripening.

MATERIALS AND METHODS

Site and plant material

This study was conducted at an avocado commercial orchard at Nico Swart estate (25° 04' 12.7" S 31° 00' 35.8" E), Kiepersol, Mpumalanga, South Africa. In the area, the average yearly temperature is 22.26 °C and the average rainfall is < 667 mm. Fruit were harvested from 11 years old 'Hass' avocado trees at different harvest maturity stage based on dry matter); early maturity (≈ 21% dry matter), mid-harvest (≈ 28% dry matter) and late harvest (≈ 35% dry matter). During these three-harvest maturities, fruit were immediately transported to the Agriculture Research Council-Tropical and Subtropical Crops (ARC-TSC) postharvest laboratory (25° 27' 04.6" S 30° 58' 09.1" E), Nelspruit, Mpumalanga, South Africa for storage and analysis.

Postharvest experimental design and treatment

The experimental design was carried out as 3 × 2 factorial factors A (early, mid- and late harvest maturity) and factor B (1-MCP at 300 g mol^{-1} and control), arranged in a completely randomized design (CRD) and replicated three times. Fruit were sorted, graded and then packed into avocado crates each containing 30 fruit, therefore, divided into six sample groups, each treatment had three replicated at each harvest maturity. Three groups were untreated and served as control [early (3 × 30 fruit), mid-(3 × 30 fruit) and late (3 × 30 fruit)]. The other three sample groups were treated with 1-MCP [early (3 × 30 fruit), mid-(3 × 30 fruit) and late (3 × 30 fruit)] at 300 g mol^{-1} in a closed plastic container for 16 hours. All six sample groups were cold stored at 5.5 °C for 28 days. After removal from cold storage, fruit were ripened at 21 °C. During ripening fruit were sensory evaluated every second day until they reached 'eat ripe' firmness. Fruit quality evaluated includes firmness, colour (subjective and objective colour parameters), and 5 fruit per treatment were sampled, freeze-dried in liquid nitrogen and subsequently, cold-stored at -21 °C for further analysis of total carotenoids and chlorophyll-*a* and-*b*, total anthocyanin and cyanidin 3-*O*-glucoside.

Determination of physicochemical parameters

Fruit firmness

Fruit firmness was determined using non-destructive digital bench top Sinclair IQ™ desktop automated machines (51DFTB, International LTD, Jarrold, Bowthorpa, Nonwich, NR5, 9.D, England). Fruit were measured three times along the equatorial region and values were expressed in Newton (N).

Subjective and objective exocarp colour parameters

Avocado fruit 'Hass' exocarp colour change was determined subjectively using eye colour change (1 – emerald-green, 2 – forest-green, 3 – olive-green, 4 - violet; 5 - purple, and 6 – black) as previously described by Mathaba et al. (2015). The same fruit samples were also used for objective colour assessment using Minolta chromameter (Model: CR-400, Minolta, Sensing Incorporation, Japan) with a white calibration plate (Y = 87.00; x = 0.3146; y = 0.3215) L^* = lightness, a^* = greenness/redness and b^* = yellowness/blueness and thereafter, converted to chroma and hue angle ($h°$) using the necessary equations according to McGuire (1992).

Total carotenoids, chlorophyll-a and chlorophyll-b

Chlorophyll and total carotenoids were determined using a UV-visible spectrophotometer as previously described by Lichtenthaler (1987). Freeze-dried 'Hass' avocado exocarps tissue 0.5 g was extracted with 10 ml of 80% acetone. The extraction tubes were kept on ice for 30

minutes and thereafter, vortexed for 30 seconds and centrifugation at 2500 × g for 5 minutes. The absorbance values of the supernatant were measured at 470, 646 and 663 nm. Calculation of content of chlorophyll-a and -b and carotenoids were as follows.

$$C_a = 12.25\ A_{663} - 2.79\ A_{646} \quad (1)$$

$$C_b = 21.50\ A_{646} - 5.10\ A_{663} \quad (2)$$

$$C_{x+c} = (1000\ A_{470} - 1.82\ Ca - 85.02\ C_b) / 198 \quad (3)$$

Where C_a and C_b represents chlorophyll *a* and *b*, and C_{x+c} total carotenoids

Total anthocyanin and cyanidin 3-O-glucoside

Extraction was done according to Cox et al. (2004), avocado exocarp tissues were milled to powder under liquid nitrogen and 0.5 g was extracted with 5 ml of 10% acetic acid/methanol (v/v) at room temperature. The extract was centrifuged at 3000 × g for 10 min, the supernatant was diluted 1:1 with methanol: water: acetic acid (50:50:10, v/v/v). The pH differential method previously described by Giusti & Wrolstad (2001) was used to determine total anthocyanin content. The diluted 1:1 supernatant was filtered through 0.45 µm nylon filters into clean vials and diluted with 1 µl of potassium chloride buffer ($pH_{1.0}$) and sodium acetate buffer ($pH_{4.5}$), separated in triplicate. The mixtures were allowed to settle in the dark for 10 minutes subsequently, absorbance values of each buffer mixture were measured at 530 and 700 nm in a UV-visible spectrophotometer. The total anthocyanin was calculated using the equation.

$$A = (A_{510} - A_{700})\ pH\ _{1.0} - (A_{510} - A_{700})\ pH\ _{4.5} \quad (4)$$

$$\text{Total anthocyanin (mg/ml)} = (A \times MW \times DF) / (\varepsilon \times L) \quad (5)$$

Where A = Absorbance, ε = Cyd-3-glucoside molar absorbance (26,900), MW = anthocyanin 164 molecular weight (449.2), DF = dilution factor, L = cell path length (1 cm).

Furthermore, using the above-described extraction method cyanidin 3-*O*-glucoside concentration was measured by HPLC as previously described by Cox et al. (2004). The HPLC system was equipped with JASCO units (LG-980-02 ternary gradient controller, AS-950 auto sampler, and a UV-975 UV/Vis detector). The chromatography column was a Phenomenex AQUA 5u C18 125A 5 um PR-18e 4.6 × 150 mm (California, United States of America), maintained at 35 °C. Where mobile phase (A) 1.5% $H_3\ PO_4$ and (B) acetic acid: acetonitrile: $H_3\ PO_4$: water (20: 24: 1.5: 54.5, v/v/v/v) was used. The solvent program started with solvent (B) at 20%, increasing to 70% after 25 minutes then 90% at 30 minutes. After 35 minutes the solvent composition was returned to the initial 20% solvent (B) and ready for the next injection. The sample injection volume was 2 µl and detection was at 530 nm.

Exocarp chilling injury

Exocarp chilling injury was measured according to the International Avocado Quality Manual (White et al., 2009) where a benchmark percentage and severity were derived as level 1= 10%; 2 = 30% and 3 = 50% chilling severity. The chilling injury was recorded only on the second day of fruit ripening as previously described by Mathe et al. (2018).

Statistical analysis

Statistical analyses were carried out using statistical software (GenStat, version 16th, VSN International, UK) and means separated using Duncan multiple range tests (DMRT) at the 5% level of significance. Furthermore, data were subjected to principal component analysis (PCA) using Unscrambler version 9.8 (Camo Process AS, Oslo, Norway). In addition, the relationship between the measured postharvest fruit quality parameters were determined by subjecting data to Pearson correlation test in Statistix software version 10.1.

RESULTS

Fruit firmness

The firmness of avocado 'Hass' fruit declines continuously during ripening, with maturity and 1-MCP treatment contributing significantly ($p < 0.05$) (Fig. 1). This study found that early harvest fruit ripened steadily thus, taking longer to ripen when compared to mid- and late harvest. Moreover, 1-MCP treated fruit ripened slowly for early and mid-harvested when compared with late-harvested fruit (Fig. 1). The ripening patterns of late harvest 1-MCP and untreated fruit were comparable, but 1-MCP treated fruit extended their ripening by 2 days.

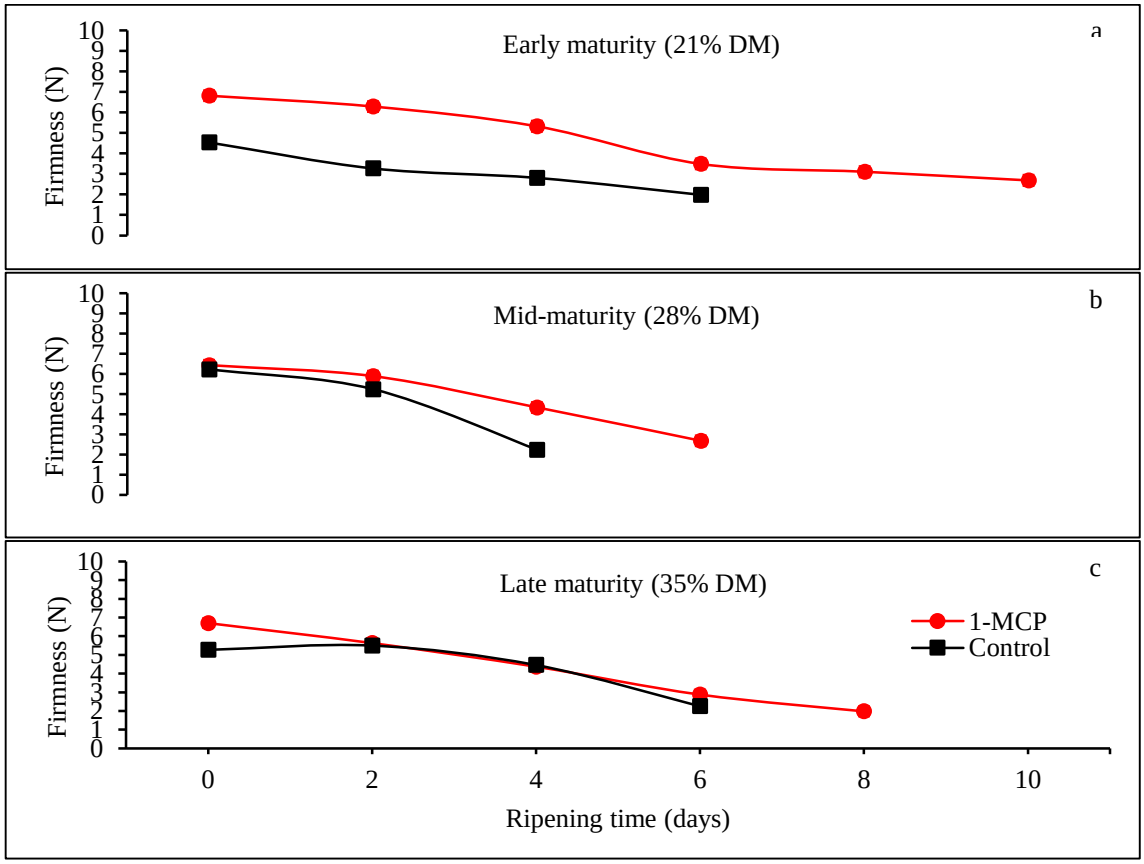

Fig. 1. Changes in fruit firmness of 'Hass' avocado fruit during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

In this study, Pearson correlation was conducted to examine the relationship between firmness and visual colour and objective colour parameters during ripening (Table 1). According to Pearson correlation, firmness and visual colour were significantly correlated with each at early, mid and late harvest (R^2 = -0.953**, -0.894** and -0.928**, respectively) for untreated fruit. For 1-MCP treated fruit, a significant negative correlation was observed at early (R^2 = -0.833**), mid (R^2 = -0.944**) and late-harvest (R^2 = -0.911**). There was a significant positive correlation between objective colour parameters (L^*, C^* and $h°$) and firmness throughout harvest time and treatments (1-MCP and untreated) during ripening. In the early season, 1-MCP fruit were significantly different from untreated fruit, especially in terms of lightness (L^*) and chroma (C^*) (Fig. 3a and Fig. 4a). The exocarp colour of 'Hass' avocado fruit showed no significant differences at late harvest, therefore, the effect of 1-MCP treatment was not significant when compared with the untreated fruit for all objective colour parameters (L^*, C^* and $h°$).

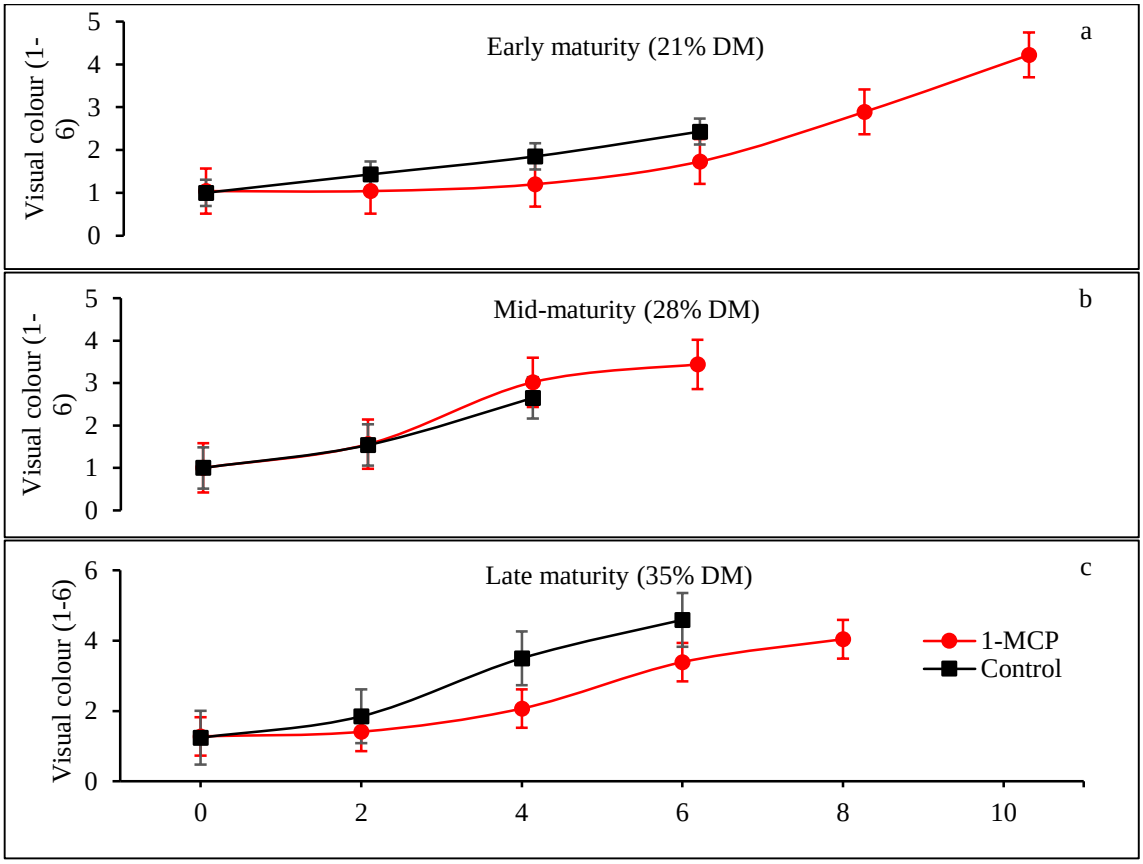

Fig. 2. Change in subjective colour (visual colour rating) of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

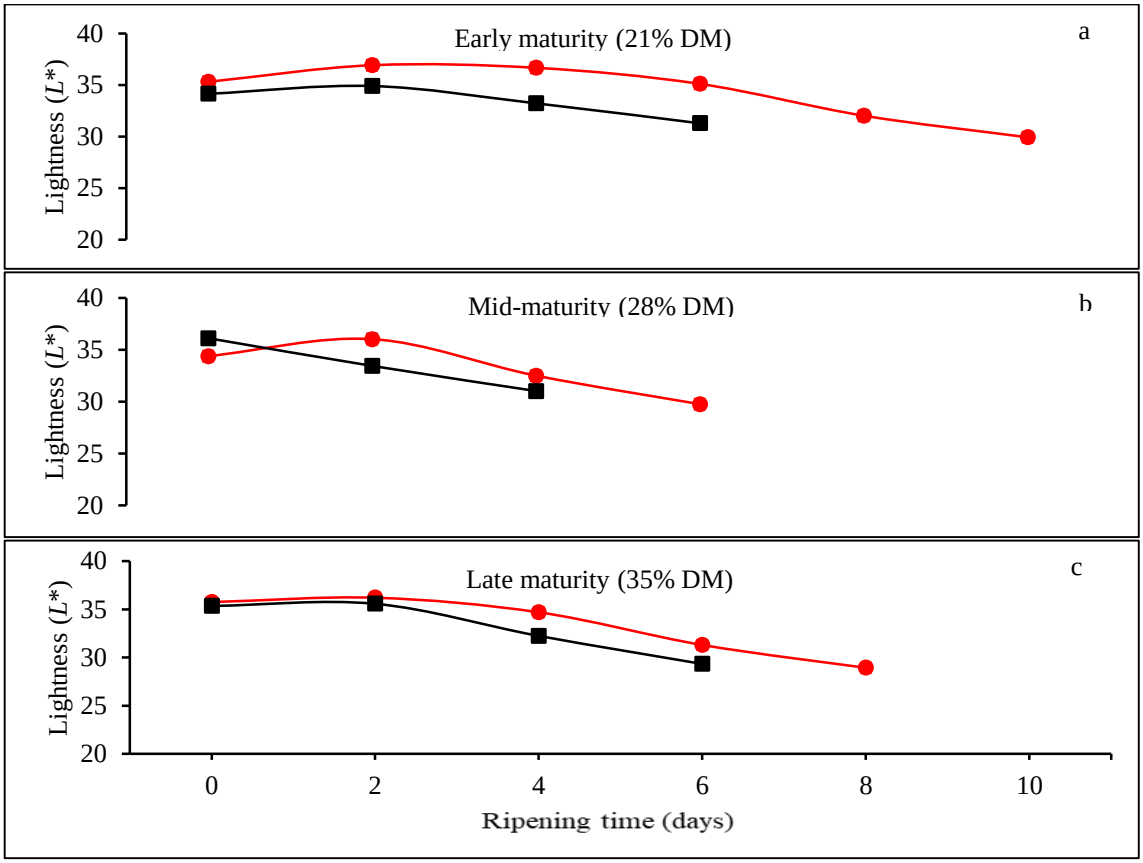

Fig. 3. Change in lightness-*L** of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

Furthermore, colour intensity was evident by the decline in objective (*L**, *C** and *h**) values, irrespective of treatment (Fig. 3, Fig. 4 and Fig. 5). In general, objective (*L**, *C** and *h*°) values decreased with days to ripening in all investigated treatments. A decrease in *h*° values reflected a change in exocarp colour from emerald-green to purple, then black, and a decline in *L** and *C** values reflected a decrease in colour intensity, resulting in a darker colour. In the present study, exocarp *h*° colour negatively correlated with cyanidin 3-*O*-glucoside at early (R^2 = -0.731**), mid (R^2 = -0.796*) and R^2 = -0.839**) for untreated fruit, and early (R^2 = -0.857**), mid (R^2 = -0.727**), late (R^2 = -0.735**) for 1-MCP treated fruit (Table 1). According to these observations, the colour of 'Hass' avocado fruit during ripening was associated with cyanidin 3-*O*-glucoside accumulation, which was increased by late harvest and 1-MCP treatment.

Subjective and objective exocarp colour parameters

In this study, the subjective exocarp colour parameters visual colour (Fig. 2) and objective (*L**, *C** and *h*°) (Fig. 3, Fig. 4, and Fig. 5) changed for all investigated treatments during ripening. It appeared that visual colour significantly ($p < 0.05$) increased due to a combined effect of fruit maturity and 1-MCP treatment on visual colour (Fig. 2). Late harvested fruit recorded higher visual colour rating when compared with early and mid-season, consequently, the fruit showed purplish to purplish black exocarp colour after reaching 'eating ripe' firmness.

Table 1. Pearson correlation coefficient between objective (Minolta chromameter values; *L**, *C**, *h°*) and subjective (visual colour rating) of 'Hass' avocado fruit exocarp colour measurement/firmness and total anthocyanin and cyanidin 3-*O*-glucoside concentrations in response to harvest maturity and 1-MCP treatment during ripening at 21 ± 2 °C.

Correlations	Early maturity		Mid-maturity		Late maturity	
	Control	1-MCP	Control	1-MCP	Control	1-MCP
	R^2		R^2		R^2	
Firmness × Vis colour	-0,953**	-0,833**	-0,894**	-0,944**	-0,928**	-0,911**
Firmness × *L**	0,703**	0,768**	0,917**	0,885**	0,944**	0,899**
Firmness × *C**	0,971**	0,912**	0,846**	0,965**	0,822**	0,959**
Firmness × *h°*	0,918**	0,841**	0,719*	0,798**	0,828**	0,897**
Firmness × Total anthoc	-0,937**	-0,827**	-0,958**	-0,946**	-0,925**	-0,753**
Firmness × Cyan 3-gluc	-0,686**	-0,665**	-0,957**	-0,965**	-0,926**	-0,858**
Vis colour × *L**	-0,788**	-0,959**	-0,890**	-0,849**	-0,956**	-0,935**
Vis colour × *C**	-0,961**	-0,955**	-0,856**	-0,989**	-0,953**	-0,968**
Vis colour × *h°*	-0,958**	-0,989**	-0,920**	-0,945**	-0,964**	-0,943**
Total anthoc × Vis colour	0,944**	0,914**	0,916**	0,824**	0,829**	0,769**
Total anthoc × *L**	-0,813**	-0,814**	-0,858**	-0,838**	-0,885**	-0,804**
Total anthoc × *C**	-0,963**	-0,875**	-0,770**	-0,870**	-0,679*	-0,733**
Total anthoc × *h°*	-0,851**	-0,712**	-0,817**	-0,623*	-0,673*	-0,603*
Cyan-3-gluc × Vis colour	0,793**	0,900**	0,882**	0,898**	0,936**	0,883**
Cyan-3-gluc × *L**	-0,795**	-0,900**	-0,827**	-0,903**	-0,942**	-0,895**
Cyan-3-gluc × *C**	-0,687**	-0,859**	-0,705*	-0,922**	-0,851**	-0,863**
Cyan-3-gluc × *h°*	-0,731***	-0,857**	-0,796*	-0,727**	-0,839**	-0,735**
Total anthoc × Cyan-3-gluc	0,787**	0,834**	0,933***	0,921**	0,894**	0,866**

Vis colour = visual colour, *L** = lightness, *C** = chroma, *h°* = hue angle, Total anthoc = Total anthocyanin, Cyan 3-gluc = Cyanidin 3-*O*-glucoside, *significant different at *p ≤ 0.05, **p ≤ 0.01, and ns = non-significant.

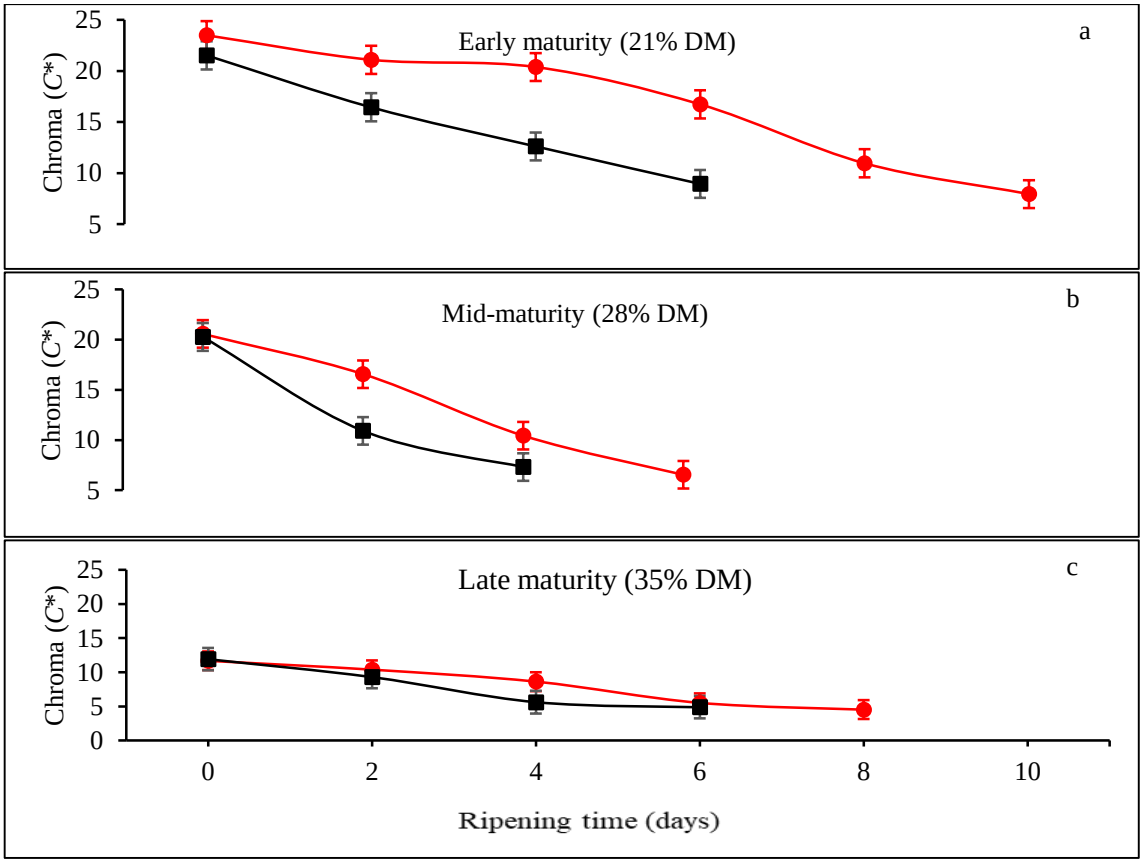

Fig. 4. Change in chroma-*C** of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

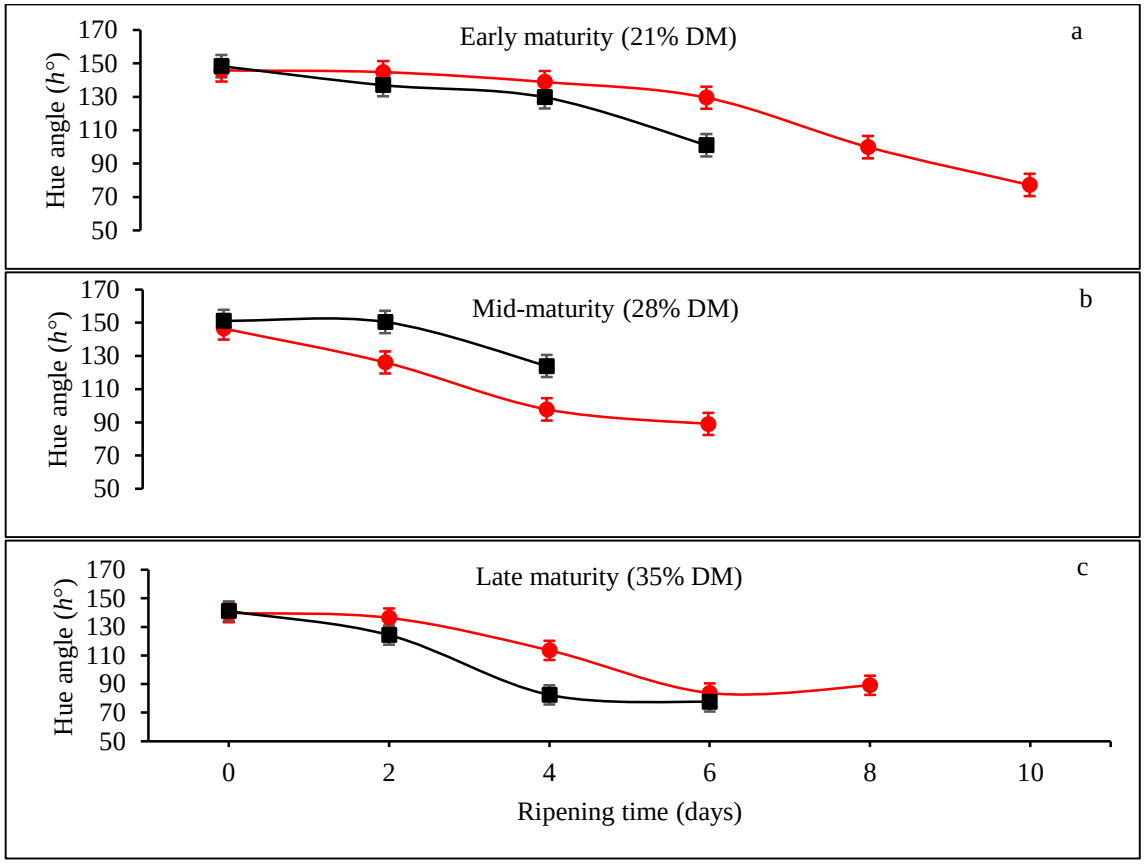

Fig. 5. Change in hue angle-*h*° of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 3).

Total carotenoids (TC), chlorophyll-a and chlorophyll-b

There was a continuous accumulation of total carotenoids, concurrently with a reduction of chlorophyll-*a* and chlorophyll-*b* content in both untreated and 1-MCP-treated fruit during all harvest maturity (Fig. 6, Fig. 7, and Fig. 8). However, during early and mid-harvest, total carotenoids and chlorophyll-*a* and -*b* contents did not decrease significantly during ripening when compared with late-harvest time. In this study, chlorophylls-*a* and -*b* content decreased in both untreated and 1-MCP treated fruit at all fruit maturities, indicating exocarp green colour degradation as the fruit ripens (Fig. 7 and Fig. 8). Furthermore, control fruit harvested late recorded the lowest total carotenoids and chlorophyll-*a* and -*b* content when compared with 1-MCP treated fruit during ripening.

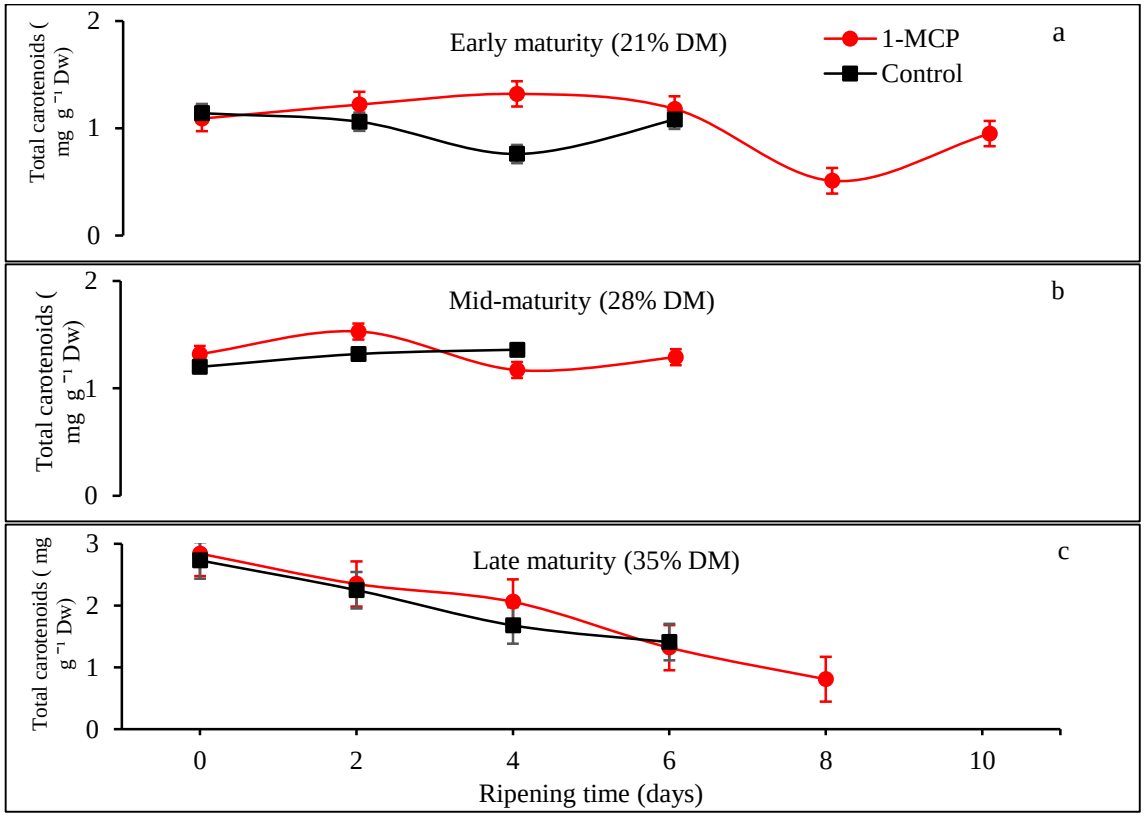

Fig. 6. Change in total carotenoids of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

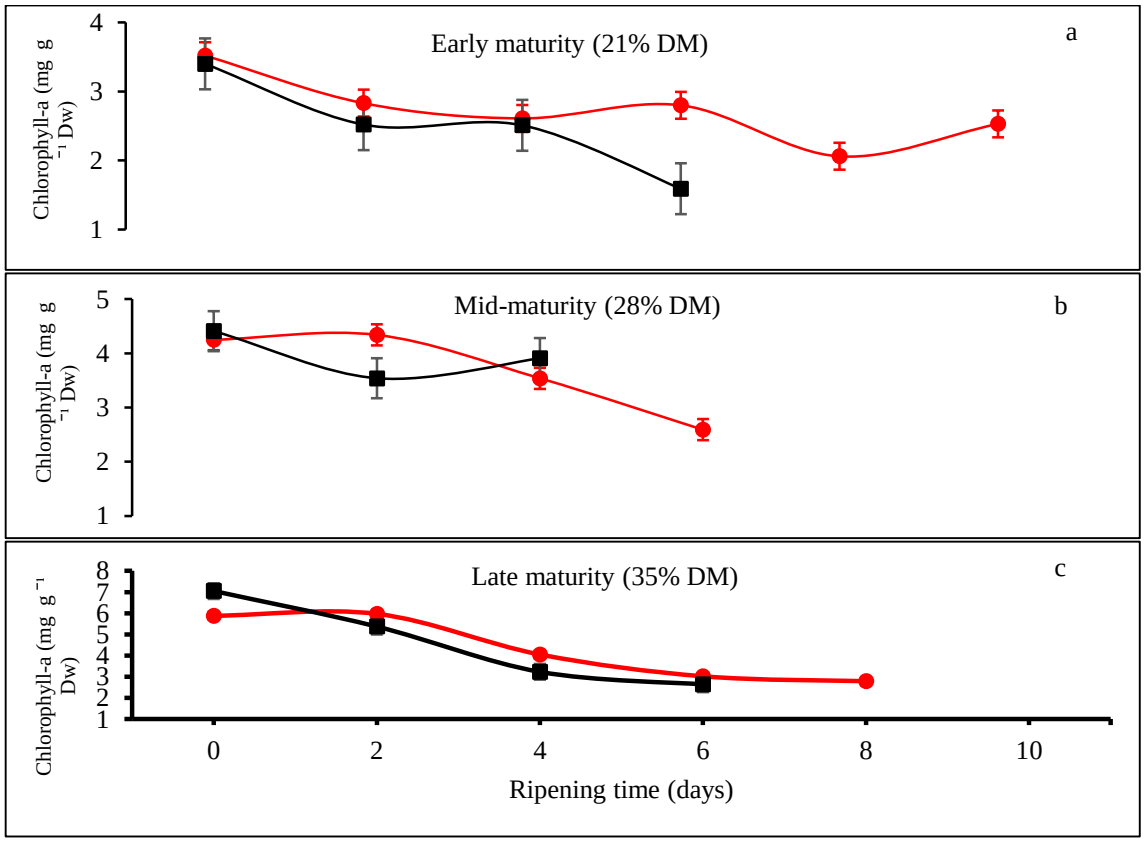

Fig. 7. Change in chlorophyll-a of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

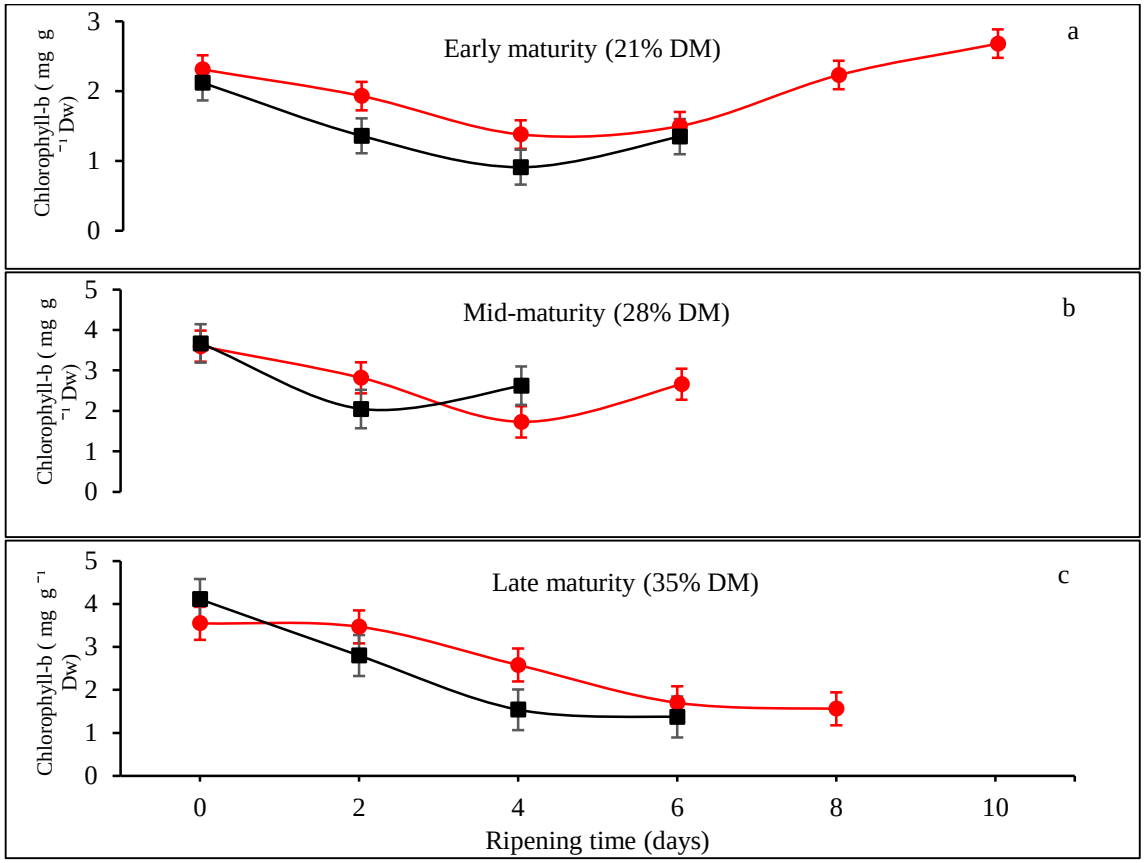

Fig. 8. Change in chlorophyll-b of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

Total anthocyanin (TA) and cyanidin 3-*O*-glucoside (Cy3G)

This study found that late-harvest fruit had higher total anthocyanin and cyanidin 3-*O*-glucoside when compared with early and mid-harvested fruit (Fig. 9 and Fig. 10). In terms of treatment effect, 1-MCP fruit showed higher total anthocyanin and cyanidin 3-*O*-glucoside accumulation on the final day of ripening, irrespective of maturity. According to the Pearson correlation, exocarp total anthocyanin concentration positively correlated with cyanidin 3-*O*-glucoside at early (R^2 = 0.787**), mid (R^2 = 0.933**) and late (R^2 = 0.894**) for untreated fruit, and early (R^2 = 0.834**), mid (R^2 = 0.921**) and late (R^2 = 0.866**) for 1-MCP treated fruit (Table 1).

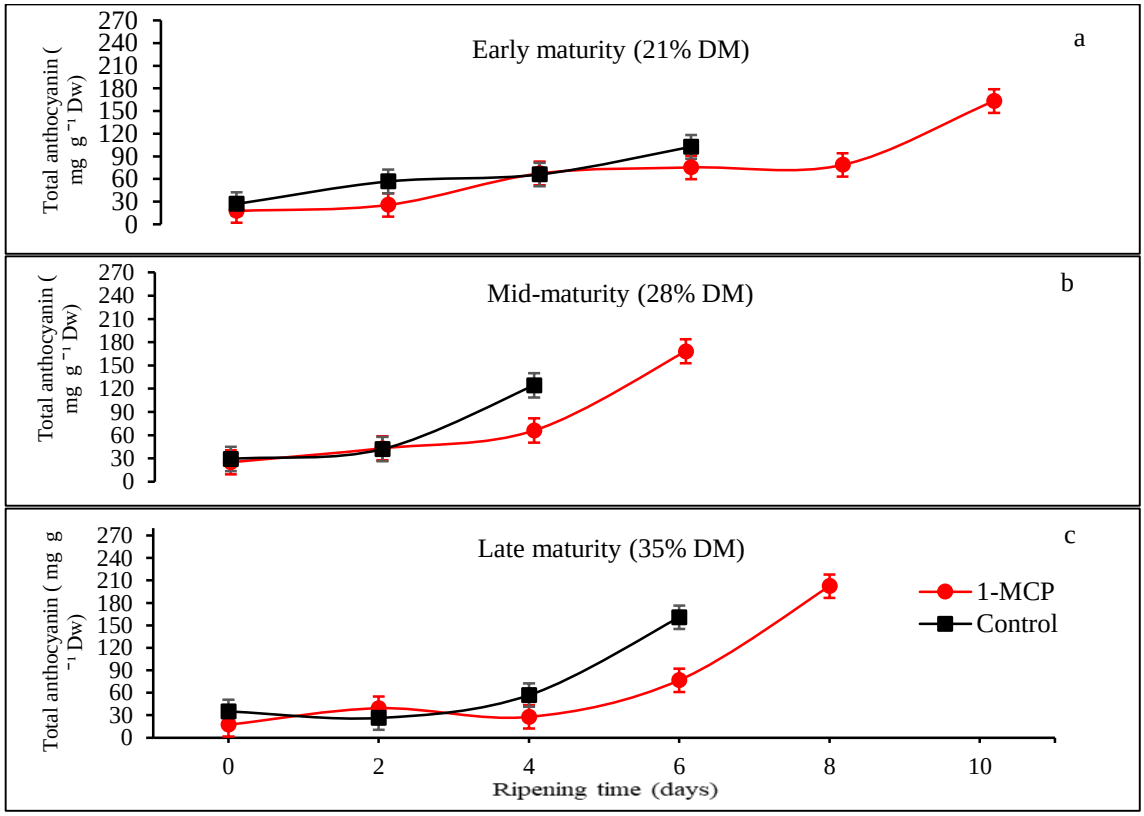

Fig. 9. Change in total anthocyanin of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

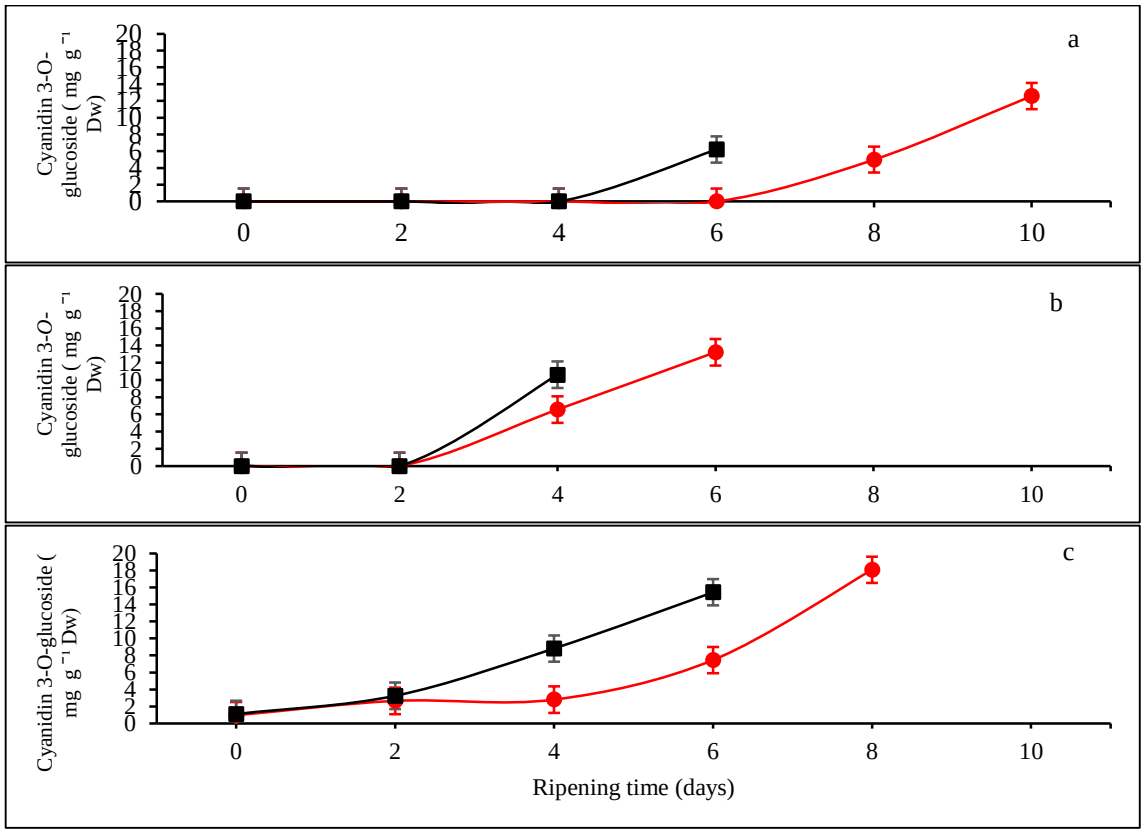

Fig. 10. Change in cyanidin 3-*O*-glucoside of 'Hass' avocado fruit exocarp during ripening at 21 ± 2 °C. Vertical bars represent standard error of means (±SE, n = 5).

Exocarp chilling injury Index (CII)

In this study, chilling injury (CI) incidence decreased with advanced fruit maturity (Fig. 11). The chilling injury index (CII) significantly ($p < 0.05$) decreased during ripening as a result of an interactive effect between fruit maturity and 1-MCP (Fig. 11). Fruit harvested early was highly susceptible to chilling injury, which resulted in a higher proportion of poor coloured fruit than those harvested mid or late. The CI symptoms in untreated fruit were higher when compared with 1-MCP-treated fruit throughout all harvest periods. CI symptoms were significantly suppressed by 1-MCP treatment as reflected in lower CII throughout harvest.

Principal component analysis (PCA)

The interrelationships between the measured parameters concerning the effect of harvest maturity, 1-MCP and ripening time were evaluated using principal component analysis (PCA) (Fig. 12). The PCA showed a total variation of 93%, whereby PC-1 explained 8% (Component 1) while PC-2 explained 85% of the observed variation. It was used to describe the interrelationship of the exocarp colour parameters to fruit ripening in response to maturation, 1-MCP treatment and ripening time (Fig. 12). In this case, the first principal component (PC-1) explained 8% of the total variation and was related to total anthocyanin, cyanidin 3-*O*-glucoside, visual colour, lightness, and firmness. The second principal component (PC-2) explained 85% of the total variation and mainly related to the hue angle.

In addition, fruit ripening characteristics were grouped with the PCA, into seven distinct clusters (Fig. 12). The first cluster included firm, emerald-green fruit that had been treated with 1-MCP at day 0, early harvest 1-MCP treated after day 2, mid-harvest control at day 0 and late harvest control at day 0. The second cluster consisted of firmer and showing forest green colour, which included, early harvest control after day 2, mid-harvest 1-MCP treated at day 2, late 1-MCP treated at day 4 and late harvest control after day 2. The third cluster consisted of fruit which were half-ripe but showed forest green colour; and included early harvest control at day 4 and early season 1-MCP treated at day 6. The fourth cluster consisted of fruit that were half-ripe and showing olive-green colour (early 1-MCP treated day 8). The fifth cluster included those that were half-ripe and showing purple colour (mid-harvest 1-MCP treated after day 4, early 1-MCP treated after day 10 and late 1-MCP treated after day 6). The sixth cluster included avocado fruit that reached eat-ripe and showed purple and black exocarp colour (late 1-MCP treated after day 8, early 1-MCP treated after day 10, late control day 6 and early control day 6). In the seventh cluster, fruit were overripe and showed black exocarp colour (mid-control after day 4 and early control after day 6). According to these clusters, 1-MCP treated fruit showed purple and black exocarp colour upon reaching ripe firmness when compared to untreated fruit, irrespective of fruit maturity.

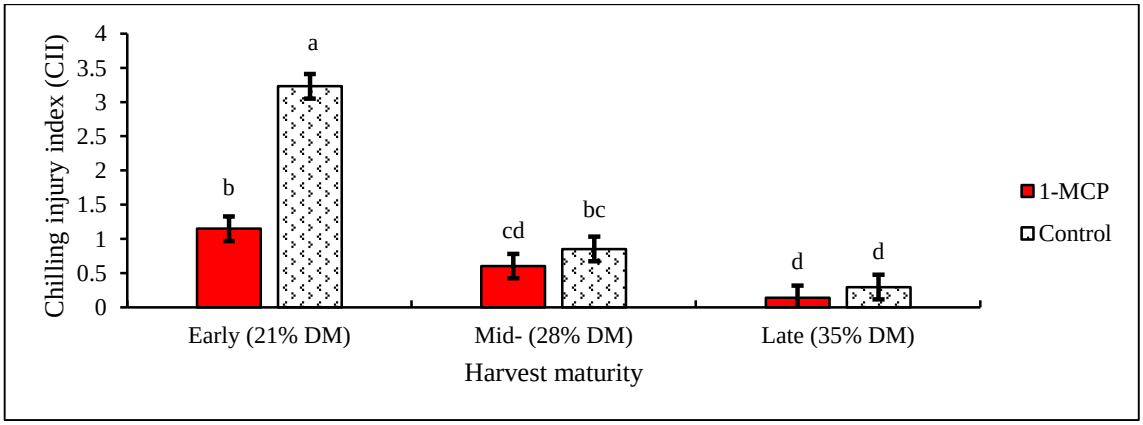

Fig. 11. Chilling injury index (CII) of 'Hass' avocado fruit during ripening at different harvest seasons. Each bar represents means of three replications and vertical bars represent standard error (±SE, *n = 30*).

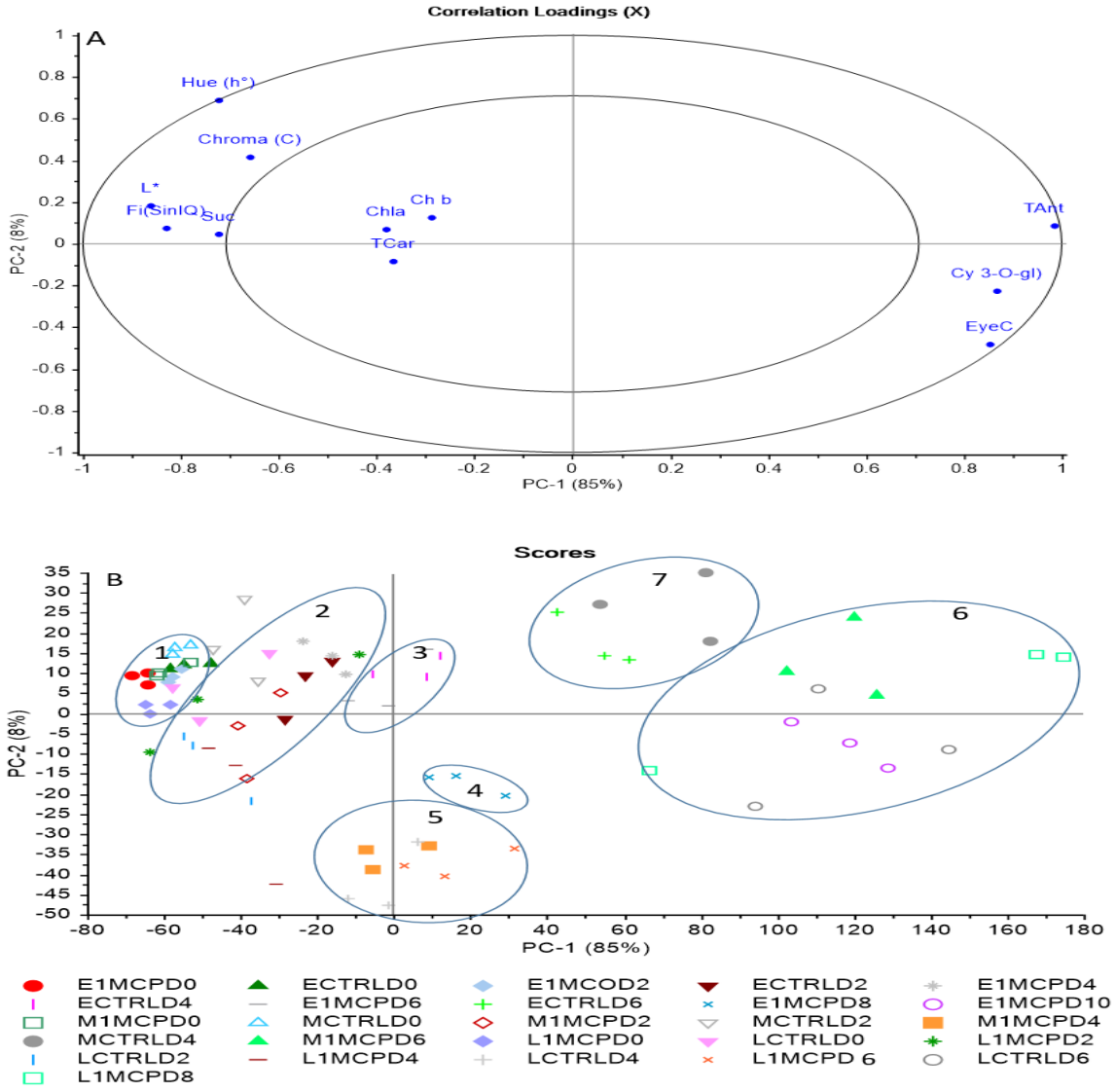

Fig. 12. Principal component analysis (PCA) showing correlation loadings. **A:** Score plot on colour physicochemical properties **B:** Score plot for the groups of 'Hass' avocado fruit exocarp colour in response to maturity and 1-MCP treatment during ripening.

DISCUSSION

The maturity of avocado fruit plays an important role in softening during ripening (Kassim et al., 2013). A study by Zauberman et al. (1977) found that fruit ripening rate was highly dependent on fruit maturity and observed that fruit harvested at early season took longer to soften when compared with fruit harvested at late maturity. The present study also found that early matured fruit ripened slower, therefore, took longer to ripen when compared with mid- and late-matured fruit (Fig. 1). Similarly, Vuthapanich et al. (1995) also reported that fruit harvested late ripened rapidly, especially when grown in warmer regions. The same study further showed that the time to ripening was influenced by moisture content (Vuthapanich et al., 1995). In this study, 'Hass' avocado fruit quality was improved during ripening following 1-MCP application, presumably due to a delay in fruit softening. Thus, 1-MCP treated fruit ripened slowly for early and mid-harvested when compared with late-harvested fruit. These results concur with observations reported by Feng et al. (2000) and Jeong et al. (2002), whereby 1-MCP treatment delayed ethylene-induced fruit softening on avocado fruit during ripening. The results suggest that late harvested fruit has high enzyme activity for ethylene

biosynthesis and high ethylene production, resulting in reduced 1-MCP effectiveness (Satekge & Magwaza, 2022).

In general, fruit softening has been reported to be resultant of cell wall degradation by enzymes such as polygalacturonase (PG), pectate lyase (PL), pectin methylesterase (PME), cellulose and β-galactosidase (Payasi et al., 2009). These enzymes are operative by breaking down the cell wall polysaccharides, which cause the pectin and hemicellulose to solubilize and depolymerize, subsequently, leading to cell wall loosening (Wakabayashi, 2000). Studies further showed that the activities of PG, PME and cellulose enzymes are highly dependent on ethylene production. For instance, softness inhibition in transgenic melon fruit was restored following treatment with exogenous ethylene (Nishiyama et al., 2007). Therefore, the activities of these enzymes can be inhibited and delayed during ripening by 1-MCP treatment. These observations were confirmed in a study reported by Jeong et al. (2002), whereby PG activity was delayed for up to 10 days in avocado fruit treated with 1-MCP. Similarly, Nishiyama et al. (2007) found that treatment with 1-MCP completely inhibited softening in melon fruit.

Avocado 'Hass' exocarp colour development has previously been linked with fruit harvest maturity advancement (Mathaba et al., 2015). This study found that fruit harvested at mid and late maturity developed purple and black colour better than early matured fruit (Fig. 2). These observations were also reported by Donetti and Terry (2014) and Mathaba et al. (2015). Their studies found that mid-harvest fruit developed purple exocarp colour during ripening, while late harvested fruit reached the desired black colour when compared with early harvest fruit (Fig. 2). This effect was ascribed to the higher accumulation of cyanidin 3-*O*-glucoside for mid and late harvest fruit during ripening (Cox et al., 2004). In addition, early maturing fruit had lower total anthocyanin concentration, resulting in reduced accumulation of cyanidin 3-*O*-glucoside for exocarp colour development.

In this study, 'Hass' avocado fruit treated with 1-MCP showed delayed colour development during ripening when compared with untreated, regardless of fruit maturity. Although 1-MCP treatment delayed colour development, it ultimately yielded a better and improved exocarp colour for early and mid-matured fruit. Similar results were reported by Feng et al. (2000) and Jeong et al. (2002), who also found that 1-MCP treatment delayed colour change in 'Hass' avocado fruit during ripening. In general, 1-MCP application improves fruit quality by inhibiting ethylene production and delaying ripening processes such as firmness, chlorophyll degradation, anthocyanin biosynthesis and final colour development (Xu et al., 2021; Zhang et al., 2021).

In this study, the decrease in L^*, C^* and $h°$ and high visual colour rating indicated an improvement in exocarp colour from green to purple then black for 1-MCP treated fruit during ripening (Fig. 3, Fig. 4 and Fig. 5). As previously observed by Cox et al. (2004), whereby, the decrease in objective colour parameters (L^*, C^* and $h°$) indicated chlorophyll degradation, and concomitantly, anthocyanin accrued leading to 'Hass' avocado exocarp colour development during ripening. In addition, PCA 1 (85% variation) showed that total anthocyanin and cyanidin 3-*O*-glucoside were interrelated with colour change (visual rating) during ripening. This was further explained by a negative correlation obtained between colour intensity ($h°$) and cyanidin 3-*O*-glucoside at early (R^2 = -0.857**), mid- (R^2 = -0.727**) and late (R^2 = -0.735**) for 1-MCP treated fruit (Table 1).

According to studies of fruit colour changes, sucrose and glucose are responsible for regulating anthocyanin biosynthesis and cyanidin 3-*O*-glucoside accumulation (Solfanelli et al., 2006; Teng et al., 2005). Moreover, sucrose and glucose can be phosphorylated by sucrose non-fermenting-related kinase enzyme (SnRK1) and hexokinase (HXK1) to produce UDP-glucose in the cytoplasm, respectively (Peng et al., 2016). The resultant UDP-glucose interacts with MYB transcriptional factors to regulate genes encoding enzymes related to

anthocyanin biosynthesis within the flavonoid's pathways (Shin et al., 2013). In this work, the 1-MCP application supposedly suppressed ethylene production, concomitantly, delaying UDP-glucose glycosylation by SnRK1 and HXK1 during early ripening when compared with the untreated fruit (Liu et al., 2017; Shin et al., 2013; Solfanelli et al., 2006).

In 'Hass' avocado fruit, the development of CI symptoms is closely associated with poor exocarp colour change during ripening (Mathaba et al., 2015). In this study, exocarp CI symptoms development decreased with fruit maturity (Fig. 11). Therefore, early matured fruit were highly susceptible to cold chilling injury damage, as they showed higher fruit proportion with poor colour when compared with mid- and late-matured fruit (Fig. 11). In previous studies, it was established that early matured 'Hass' avocado fruit were highly susceptible to CI when compared with late matured fruit (Bower & Magwaza, 2004; Faubion et al., 1992; Mathaba et al., 2015). Furthermore, Mathaba et al. (2015) reported that CI development was closely associated with exocarp colour de-synchronization with softening in early harvested fruit than late during ripening. In addition, control fruit showed higher CI symptoms when compared with 1-MCP treated fruit during ripening. Previous studies on 'Hass' avocado fruit also reported a similar trend (Hershkovitz et al., 2005; Jeong et al., 2002; Pesis et al., 2002).

Additionally, ethylene production is arguably involved in chilling injury development during ripening (Hong & Gross, 2000; Cocetta & Natalin, 2022). The production of reactive oxygen species (ROS) induced by chilling storage stress reduced membrane integrity, increase lipid peroxidation damage and molandialdehyde (MDA) accumulation, subsequently, leading to the development of chilling injury symptoms (Endo et al., 2019). However, 1-MCP treatment alleviates chilling induced mesocarp discolouration and CI symptoms development in avocado fruit (Pesis et al., 2002). Our results in the current study supported these findings, whereby, 1-MCP treatment significantly suppressed CI symptoms as indicated by a lower CI index throughout fruit maturity stages (Fig. 11). Another study supporting these observations showed that 1-MCP treatment prior to cold-storage reduced the CI incidence in 'Hass' avocado fruit (Hershkovitz et al., 2005). Woolf et al. (2005), however, did not find that 1-MCP could reduce external chilling injury at 0 °C. It has been reported that chilling injury is associated with reactive oxygen species (ROS) in the exocarp of the 'Hass' avocado fruit (Pesis et al., 2002).

Tesfay et al. (2011) found that exposure of 'Hass' avocado fruit to oxidative stress such as chilling temperature-induced ROS production, subsequently leading to oxidative damage. In avocado fruit, the phenols merged with antioxidants are the major defence systems responsible for scavenging ROS, thereby, protecting the cells against oxidative stress (Tesfay et al., 2010). Avocado fruit exocarps are rich in phenolic compounds that contribute to their antioxidant properties (Rodríguez-Carpena et al., 2011; Wang et al., 2010). Catechin and epicatechin phenols dominate the antioxidant system (Tesfay et al., 2009). Therefore, the ability of avocado exocarp to tolerate oxidative stress is highly dependent on its ability to produce catechin and epicatechin (Tesfay et al., 2011).

Furthermore, the exposure of avocado fruit to chilling stress results in an up-regulation of phenylpropanoid, which is an important pathway for the synthesis of both the phenols and anthocyanin pigment (Mahattanatawee et al., 2006). In general, phenylalanine ammonia-lyase (PAL) enzyme is a rate-controlling enzyme in phenylpropanoid pathway leading to phenols and anthocyanin synthesis (Villa-Rodríguez et al., 2011). Studies further showed that PAL enzyme activity is highly dependent on ethylene production (Martinez & Whitaker, 1995). Therefore, the potential benefit of postharvest 1-MCP treatment is to delay ethylene production during chilling stress. Physiologically, delayed ethylene synthesis and action down-regulate PAL activity, thereby delaying phenols synthesis and their antioxidant capacity during cold storage. This was supported by Zhang et al. (2013), who found changes in the proportion of antioxidant parameters in 'Booth 7' avocado fruit were delayed subsequent to 1-

MCP treatment due to suppression of ethylene production and fruit softening. Consequently, ethylene production increase the activity of PAL, subsequently, leading to the synthesis of phenols, as a result, scavenging ROS, therefore, reducing the development of exocarp CI symptoms during ripening (Fawbush et al., 2009; Zhang et al., 2013). In addition, sugars and antioxidants protect the chlorophyll systems against chilling damage during cold storage, while 1-MCP reduce respiration rate, inhibit ethylene production, and delay chlorophyll degradation during ripening (Lv et al., 2020).

In general, chlorophyll degradation involves chlorophyll carbolic enzymes such as chlorophyllase and Mg-dechelatase (Cheng et al., 2012). Alternatively, chlorophyll degradation could also be triggered through oxidative stress induced by ROS, particularly peroxidise (POD) (Kariola et al., 2005). Sharma et al. (2012) reported that excess ROS result in lipid peroxidation, protein denaturation and DNA damage. In addition, ROS acts as a signal transduction messenger triggering protective response through the accumulation of carotenoids and polyphenols compounds (Lukaszewicz et al., 2004). Frequently, the action of ROS results in PAL promoting enzymes, consequently, synthesis of antioxidant compounds such as phenols, phenolic acid and anthocyanin pigments (Karuppanapandian et al., 2011). It was reported that ROS signalling strength depends on the balance between oxidant production and removal by antioxidant properties (Theocharis et al., 2012).

During normal chlorophyll degradation, ROS are generated at a low level, which constitutes a balance between production and removal (Sharma et al., 2012). The counterbalance is disrupted by chilling stress when intracellular ROS levels increase, resulting in senescence peroxidation and CI damage (Vellosillo et al., 2010). This study found that early matured fruit exhibited poor colour change due to damage to the chlorophyll system. Fruit that were mid- and late-matured were probably protected from chlorophyll degradation by high exocarp sugar concentration and accumulation of antioxidants, in particular cyanidin 3-*O*-glucoside. In contrast, early matured 'Hass' avocado fruit poor colour development can be attributed to low sugar accumulation at harvest and anthocyanin synthesis during ripening. 1-MCP treatment conserved pre-harvest sucrose, which, assumably, promoted PAL activity, leading to higher phenol antioxidant accumulation and anthocyanin pigment accumulation during ripening than untreated fruit. This resulted in improved colour change in 1-MCP fruit, regardless of its maturity. As a result of 1-MCP treatment, ethylene synthesis was suppressed, reducing ROS formation, and protecting the chlorophyll system (chlorophyll-*a* and -*b*) against damage during chilling stress in early and mid-matured fruit.

The PCA analysis showed that the ripening period of untreated and 1-MCP treated ‘Hass’ avocado fruit was grouped into seven clusters. These clusters represented ripening as influenced by the combined effect of firmness and exocarp colour change *viz*: unripe-emerald-green, unripe-forest green, partially ripe-olive green, ripe-purple, ripe-black and senescence-black. In this study, ripening followed a climacteric pattern; therefore, clusters 1, 2 and 3 can be grouped as pre-climacteric whereas cluster 4, 5 and 6 as climacteric and cluster 7 as senescence stage. In addition, these clusters demonstrated that colour change makes an ideal non-destructive reference for classifying fruit ripeness of ‘Hass’ avocado fruit during ripening. The difference between clusters in this study corroborated a distinctive change in cell wall enzymatic activities, anthocyanin and cyanidin 3-*O*-glucoside synthesis and sucrose degradation rate during ripening. In this study, although 1-MCP treatment delayed these physiological processes, it resulted in improved fruit quality particularly early-season when compared with untreated fruit.

CONCLUSION

The study found that different maturity stages of 'Hass' avocado fruit responded differently to 1-MCP treatment. Results showed that 1-MCP treatment significantly reduced the damage caused by CI during storage for early and mid-harvest maturities. In this study, 1-MCP treatment delayed firmness, colour, and chilling injury development for early and mid-matured fruit, resulting in improved exocarp colour development at the end of ripening. Thus, it is concluded that maturity and 1-MCP had a positive influence on avocado fruit's exocarp colour pigments (total anthocyanin and cyanidin 3-*O*-glucose) synthesis and accumulation during ripening.

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Conflict of interest
The authors declare that there is no conflict of interest.

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