https://doi.org/10.17221/72/2023-HORTSCI

Effect of ripening temperature on early-season 'Hass' avocado fruit exocarp colour development and pigmentation during ripening

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Citation: Sibeko D.S., Shikwambana K., Mathaba N. (2024): Effect of ripening temperature on early-season 'Hass' avocado fruit exocarp colour development and pigments during ripening. Hort. Sci. (Prague), 51: 152–159.

Abstract: South African 'Hass' avocado fruit harvested early season are vulnerable to colour desynchronisation with softening during ripening, reducing their international and local market aesthetic value. So far, research has proven that 'Hass' avocado fruit exocarp colour desynchronization is an early season conundrum. However, there is insufficient literature on underlying factors causing exocarp colour desynchronisation. Therefore, the study aimed to investigate the ripening temperature effect on early season 'Hass' avocado fruit exocarp colour development and pigments during ripening. Early season 'Hass' avocado fruit were stored at 5.5 °C for 28 days thereafter, ripened at 16, 21 and 25 °C and evaluated for firmness, visual and objective colour (L^*, C^* and $h°$), total chlorophyll and anthocyanin content. The results showed that the visual colour of fruit ripened at 25 °C was significantly higher compared to 21 and 16 °C after 2 and 4 ripening days. This study found that ripening temperature had no significant influence on chlorophyll degradation. However, fruit ripened at 25 °C accumulated significantly higher exocarp anthocyanin concentration compared to lower ripening temperatures. In conclusion, ripening early season 'Hass' avocado fruit at 25 °C resulted in improved exocarp colour development and anthocyanin accumulation.

Keywords: anthocyanin; chlorophyll; chromaticity; firmness; visual colour

The 'Hass' avocado (*Persea americana* Mill) fruit is characterised by an exocarp colour change from green to purple-black during ripening (Cox et al. 2004). The intensity of the purple-black colour is determined by chlorophyll degradation concomitant with anthocyanin synthesis in the fruit's exocarp (Cox et al. 2004; Ashton et al. 2006; Arancibia-Guerra et al. 2022). However, 'Hass' avocado fruit harvested early season remains green even after reaching edible softness (Mathaba et al. 2017). The desynchronisation of exocarp colour with softening is problematic as retailers and consumers use these quality attributes to determine when the fruit is ready to eat (Mathaba et al. 2015). Therefore, 'Hass' avocado fruit exocarp colour desynchronisation with softening could lead to consumer confusion regarding purchases. Also, postharvest losses may result from rejected fruit not meeting ready-to-eat colour standards (Nelson 2012; Munhuweyi et al. 2020). Currently, there is no effective postharvest technol-

Supported by a grant from the National Research Foundation (NRF).

https://doi.org/10.17221/72/2023-HORTSCI

ogy to minimize exocarp colour desynchronisation with softening during ripening.

Avocadoes are climacteric; thus, their ripening is associated with a peak in respiration and ethylene production (Defilippi et al. 2018; Vincent et al. 2020). The increase in respiration during ripening results in methionine being converted to S-adenosyl methionine, the precursor of 1-aminocyclopropane-1-carboxylic acid (ACC) immediate ethylene precursor (Zhang et al. 2011; Tucker et al. 2017; Vincent et al. 2020). According to Vincent et al. (2020), temperatures influence the function of ACC synthase (ACS) and ACC oxidase (ACO). Vincent et al. (2020) found that transferring 'Bacon' avocado fruit from 4 to 25 °C increased ethylene production and softening parallel to increases in ACC synthase and ACC oxidase activities. In general, ripening avocados at 25 °C induces ethylene synthesis, therefore, altering ethylene-dependent processes such as exocarp colour and mesocarp firmness (Arpaia et al. 2018; Vincent et al. 2020). More specifically, avocado fruit ripening at 25 °C triggers ethylene to stimulate fruit softening through the activation of pectin methylesterase, polygalacturonase and β-galactosidase, thus inducing the depolymerization and solubilization of pectin and cellulose in the cell wall (Defilippi et al. 2018; Vincent et al. 2020).

Earlier research has shown that the change in exocarp colour of the 'Hass' avocado and early- and medium-maturing apple cultivars is influenced by ripening temperature (Cox et al. 2004; Honda et al. 2014). These researchers found that ripening fruit at 25 °C resulted in higher anthocyanin content and better exocarp colour than at 20 and 15 °C. However, Cox et al. (2004) experimented using late-season 'Hass' avocado fruit, which has been reported as not prone to colour desynchronisation (Nelson 2012; Mathaba et al. 2015). Therefore, the observed darker exocarp colouration (Cox et al. 2004) may not be primarily induced by higher temperatures but by advanced fruit maturity as reported by Mathaba et al. (2015). Moreover, Mathaba et al. (2015) reported that avocado ripening should be limited to 16 °C because temperatures lower than that cause chilling injury to the fruit. In 'Red Beauty' plum fruit, Niu et al. (2017) found that enzymatic activities of chalcone synthase (CHS), anthocyanidin synthase (ANS), dihydroflavonol 4-reductase (DFR) and phenylalanine ammonia-lyase (PAL) involved in cyanidin 3-*O*-glucoside biosynthesis increased faster at high temperatures (25 and 35 °C) compared to 20 °C. However, Arpaia et al. (2018) reported that temperatures above 25 °C affected fruit quality by enhancing stem end rot and mesocarp darkening in comparison to 20 °C. The relationship between ripening temperature, ethylene production and anthocyanin biosynthesis appears to be closely related. Therefore, keeping avocados at normal storage conditions (21 °C) during ripening may contribute to poor anthocyanin accumulation and, consequently, desynchronisation with softening. However, there is no empirical evidence of temperature effect on anthocyanin accumulation relative to fruit softening in early-season 'Hass' avocado fruit. This study investigated the effect of ripening temperatures (16, 21 and 25 °C) on exocarp pigments and colour development of early-season 'Hass' avocado fruit during ripening.

MATERIAL AND METHODS

Plant material. Avocado 'Hass' fruit were procured from Nico Swart commercial farm in Kiepersol, Hazyview, Mpumalanga (25°04'12.7"S, 31°00'35.8"E). The farm is characterised by a warm subtropical climate with an average annual temperature of 26 °C and rainfall ≤ 667 mm. 'Hass' avocado fruit were harvested inside and outside the tree canopy from 11-year-old trees treated alike. No tree training or fruit thinning techniques were carried out throughout the season. The avocado fruit were harvested at an early maturity stage in May 2022 and transported to the University of Mpumalanga postharvest laboratory, where they were graded. Fruit of uniform size with no mechanical or pathological defects were selected. Fruit were divided randomly before cold storage and analysis.

Experimental design. The experiment design was carried out as a completely randomised design (CRD) with three replicates. Fruit were stored at 5.5 °C temperature and 85–90% relative humidity (RH) for 28 days. After 28 days, the fruit were removed from storage and held at three different ripening temperature treatments at 16, 21 and 25 °C until fully ripe. Fruit quality measurements were taken at two days intervals during ripening and three fruit samples were collected and immediately frozen in liquid nitrogen and stored at –80 °C until further analysis.

Sample preparation for pigment determination. Avocado exocarp samples were separated from sampled fruit using a peeler on each evaluation day during ripening. The exocarp samples were dried in a freeze dryer, ground into a fine powder, and stored at –80 °C until further analysis.

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Fruit firmness. Fruit firmness was measured using a non-destructive firmness tester (Bareiss HP-FFF, Germany). Each fruit was measured at three points along the central axis of the fruit. Units were expressed as newton (N).

Exocarp colour change. Exocarp colour change of 'Hass' avocado fruit was determined subjectively using visual colour rating: 1 – emerald green; 2 – forest green; 3 – olive green; 4 – purple; 5 – black (Figure 1; Mathaba et al. 2015) and objective colour measurement were determined using a Minolta chromameter (Model: CR-400, Minolta, Sensing Incorporation, Japan), and average readings at three points against each other on the fruit were measured. Colour chromaticity parameters inclusive [L^*– lightness (a^* and b^*); C^*– chroma; $h°$– hue angle]. Where a^* indicates greenness/redness, and b^* for blueness/yellowness, the a^* and b^* are then calibrated to C^* and $h°$ using two formulas (1 and 2) from McGuire (1992). A C^* value indicates the degree of colour saturation, whereas a $h°$ value of 270° – blue, 180° – bluish-green, 90° – yellow and 0° – red (Mathe et al. 2018).

$$C^* = (a^2 + b^2)^{1/2} \quad (1)$$

$$h° = 180° + \tan/(a^*/b^*) \quad (2)$$

Total chlorophyll and carotenoid content. Using a method described by Shikwambana et al. (2021), ground exocarp powder (1 g) was added to 10 mL of 80% (v/v) cold acetone for 30 minutes, the mixture was thereafter centrifuged (Model: Z206A, Hermle Labortechnik GmbH, Germany) at 6 000 × g for 5 minutes. Subsequently, the supernatants were read using a spectrophotometer (UV- Shimadzu, Corp. Japan) at 663 nm, 646 nm and 470 nm. The total chlorophyll and carotenoid were calculated using the following formulas:

$$\text{Ca} = A_{663} \times 12.25 - A_{646} \times 2.79 \quad (3)$$

$$\text{Cb} = A_{646} \times 21.50 - A_{663} \times 5.10 \quad (4)$$

$$\text{Total chlorophyll} = 20.2 \times A_{646} + 8.02 \times A_{663} \quad (5)$$

$$\text{Total carotenoids} = 4.37 \times A_{470} + 2.11 \times A_{663} - 9.10 \times A_{646} \quad (6)$$

where: Ca – chlorophyllide *a*; and Cb – chlorophyllide *b*

Total anthocyanins. Anthocyanins were extracted with methanol, as described by Shikwambana et al. (2021). Dried-freeze exocarp tissue powder (0.5 g) was added to a 5 mL test tube of 10% methanol (v/v) and left for 24 hours at room temperature to extract. After that, the supernatant was diluted with methanol, water, and acetic acid (50 : 50 : 10 v/v/v) before being centrifuged (Model: Z206A, Hermle Labortechnik GmbH, Germany) at 3 000 × g for 10 minutes. Then, the supernatant was filtered through 0.45 µm nylon filters into vials. Using the pH differential method (Giusti, Wrolstad 2001), two dilutions of the supernatant were prepared, one with pH 1.0 (potassium chloride 0.001 mL) buffer and the other with pH 4.5 (sodium acetate, 0.001 mL) buffer. The pH 1.0 and 4.5 buffers were used to measure the absorbance at 520 and 700 nm. After letting the mixtures settle for 10 minutes, the absorbance values of each buffer mixture were determined using a spectrophotometer (UV-1800, Shimadzu, Corp. Japan) at 520 and 700 nm.

$$\text{Total anthocyanins} = \frac{A \times \text{Mw} \times \text{Df} \times 1\,000}{\varepsilon \times l} \quad (7)$$

where: A = (510 nm – 700 nm) pH 1.0 – (510 nm – 700 nm) pH 4.5; Mw = 449.2 g/mol for cyanidin 3-*O*-glucoside (Cyd-3-glu); DF = (1), ε = 26 900 molar extinction coefficients for Cyd-3-glu; l = 1 cm

Figure 1. Visual colour rating (visual score: 1–5) of 'Hass' avocado fruit
1 – emerald green; 2 – forest green; 3 – olive green; 4 – purple; 5 – black

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Data analysis. Analysis of variance (ANOVA) was performed using GenStat version 18th statistical software. All significant pairs of treatment means were compared using the Least Significant Difference (LSD) test at a 5% probability level. Pearson's correlation was used to determine the relationship between pigments (chlorophyll and anthocyanin), colour parameters and firmness.

RESULTS AND DISCUSSION

Fruit firmness (softening). The effect of ripening temperature on early-season 'Hass' avocado fruit firmness was significant ($P < 0.001$) during ripening (Table 1). The results showed that fruit firmness was significantly higher when ripened at 16 °C than those ripened at 21 and 25 °C after 2 days of ripening. The lowest fruit firmness was recorded during ripening at 21 and 25 °C after 4 days, but the difference was not statistically significant when compared with ripening at 16 °C (Table 1). The results are comparable to Vincent et al. (2020), who reported higher firmness loss in 'Bacon' avocado fruit during ripening at 25 °C. According to Defilippi et al. (2018), several factors are associated with softening, such as increased ethylene production, respiration and cell wall pectin depolymerisation. Pedreschi et al. (2019) reported that pectin enzymes such as cellulase, pectin methyl esterase (PME) and polygalacturonase (PG) primarily constitute ripening associated with pectin degradation and softening in avocado fruit. In several climacteric crops such as 'Hom Thong' banana, 'Jonagold' apples and 'Guifei' mangoes, studies found that transcripts of PG, PME and cellulase enzymes positively correlated with fruit ripening and softening (Amnuaysin et al. 2012; Gwanpua et al. 2014; Liu et al. 2020). It has been reported that temperatures above 20 °C enhance the activities of these enzymes in 'Hass' avocado fruit (Defilippi et al. 2018). This could indicate that pectin-degrading enzymes were highly activated when the fruit ripened at 21 and 25 °C, leading to rapid ripening and softening (Table 1).

Exocarp colour. The results from this study showed that ripening temperatures significantly affected ($P < 0.05$) exocarp visual colour, lightness (L^*), chroma (C^*) and hue angle ($h°$) during ripening (Table 1). The results showed that the visual colour of fruit ripened at 25 °C was significantly higher than at 21 and 16 °C after 2 and 4 days of ripening (Table 1). According to the visual colour rating, fruit that ripened at 25 °C reached olive green (≈ 3-visual colour rating) after 2 days of ripening, while those that ripened at 21 and 16 °C only changed to forest green (≈ 2-visual colour rating). At the end of ripening (day 4), fruit ripened at 21 and 25 °C reached purple (≈ 4-visual colour rating), while those ripened at 16 °C reached forest green (3-colour- rating) (Table 1). Furthermore, chromaticity parameter values (L^*, C^* and $h°$) decreased during ripening, irrespective of ripening temperature (Table 1). These results verified previous studies- that showed a decrease in chromaticity values during 'Hass' avocado fruit ripening (Cox et al. 2004; Shikwambana et al. 2021). Moreover, the re-

Table 1. Changes in firmness, visual colour and chromaticity of 'Hass' avocado fruit during ripening. Fruit were stored at 5.5 °C for 28 days and ripened at 16, 21 and 25 °C

Ripening temperature	Ripening days	Firmness (N)	Visual colour (1–5)	L^*	C^*	$h°$
16 °C	0	9.04 ± 0.03^{a}	1 ± 0.00^{a}	36.54 ± 0.50^{a}	18.32 ± 0.87^{a}	143.44 ± 0.63^{a}
	2	8.29 ± 0.13^{b}	1.83 ± 0.07^{b}	35.02 ± 0.32^{b}	12.32 ± 0.64^{c}	110.63 ± 3.00^{bc}
	4	5.69 ± 0.16^{e}	2.93 ± 0.08^{c}	34.70 ± 0.27^{b}	4.49 ± 0.38^{e}	107.70 ± 3.31^{bc}
21 °C	0	8.99 ± 0.03^{a}	1 ± 0.00^{a}	36.38 ± 0.35^{a}	16.98 ± 0.62^{b}	142.29 ± 0.34^{a}
	2	7.74 ± 0.15^{c}	2.3 ± 0.09^{d}	33.26 ± 0.24^{c}	9.11 ± 0.62^{d}	105.15 ± 2.25^{c}
	4	4.8 ± 0.08^{f}	3.6 ± 0.09^{e}	31.00 ± 0.26^{d}	3.66 ± 0.19^{e}	119.48 ± 2.56^{b}
25 °C	0	9.06 ± 0.04^{a}	1 ± 0.00^{a}	36.64 ± 0.42^{a}	16.91 ± 0.77^{b}	142.61 ± 0.44^{a}
	2	7.40 ± 0.12^{d}	2.63 ± 0.11^{f}	32.82 ± 0.37^{c}	8.65 ± 0.64^{d}	100.08 ± 2.79^{c}
	4	4.74 ± 0.06^{f}	3.9 ± 0.06^{g}	29.92 ± 0.21^{e}	3.99 ± 0.18^{e}	84.44 ± 11.89^{d}
P-value: $T \times D$		< 0.001	< 0.001	< 0.001	0.009	0.001
$LSD_{(0.05)}$		0.279	0.186	0.721	1.275	12.194

T – temperature; D – ripening days; ± – standard error of 3 replicates; L – lightness; C – chroma; $h°$ – hue angle; $^{a–g}$indicate a significant difference in means according to the LSD test ($P \leq 0.05$)

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sults showed that fruit ripening at 25 °C had a significantly lower L^* value than values recorded at 21 and 16 °C after four ripening days (Table 1). In comparison, no significant differences in C^* were observed in all ripening temperatures after four ripening days. In terms of colour intensity ($h°$), the results indicated that fruit ripened at 25 °C showed the lowest $h°$ value than those ripened at 21 and 16 °C when fully ripe (day 4). Shikwambana et al. (2021) reported that the decrease in chromaticity values represented the formation of 'Hass' avocado fruit's purple colour due to the degradation of chlorophyll and the concurrent anthocyanin pigment accumulation.

Total chlorophyll content. Chlorophyll is responsible for the 'Hass' avocado fruit exocarp green colour, its initial concentration is high but decreases during ripening (Figure 2A). Thereafter, chlorophyll is degraded enzymatically during fruit ripening, which is accompanied by a corresponding green exocarp colour loss (Ashton et al. 2006; Kuai et al. 2018). This study found no significant differences ($P = 0.665$) in total chlorophyll between the three ripening temperatures at the end of ripening, although fruit ripened at 16 °C showed the lowest exocarp chlorophyll content when compared to 25 and 21 °C ripened fruit (Figure 2A). The present results were comparable to observations reported in earlier studies (Cox et al. 2004; Shikwambana et al. 2021). However, temperature plays a role in the breakdown of chlorophyll in other fruit crops. In 'Cavendish' banana fruits, chlorophyll degradation was studied by Yang et al. (2009) and Du et al. (2014) who found that chlorophyll degradation occurred slower when fruit were ripened at 30 °C compared to 20 °C. The suppression of essential genes for chlorophyll degradation caused lower chlorophyllase and Mg-dechelatase activities at 30 °C compared to 20 °C (Yang et al. 2009; Du et al. 2014). This suggests that the process of chlorophyll breakdown during ripening may not be as sensitive to temperature variations in 'Hass' avocados. This finding aligns with research indicating that chlorophyll degradation may be governed by factors other than temperature, such as fruits' initial content at harvest (Donetti, Terry 2011).

Total anthocyanin content. In 'Hass' avocado fruit, the purple-black colouration after initial chlorophyll degradation is linked to anthocyanin accumulation in the exocarp (Ashton et al. 2006; Arancibia-Guerra et al. 2022). This study found that ripening temperatures significantly affected total anthocyanin content ($P = 0.007$) during ripening (Figure 2B). The results are consistent with previous studies on the temperature-dependent regulation of anthocyanins in 'Hass' avocado (Cox et al. 2004); 'Yunhongli No. 1' pears (Zhang et al. 2012); 'Tsugaru Hime', 'Akane', and 'Akibae' apples (Honda et al. 2014); 'Red Beauty' plum (Niu et al. 2017); and 'Benihoppe' strawberry (Mao et al. 2022). However,

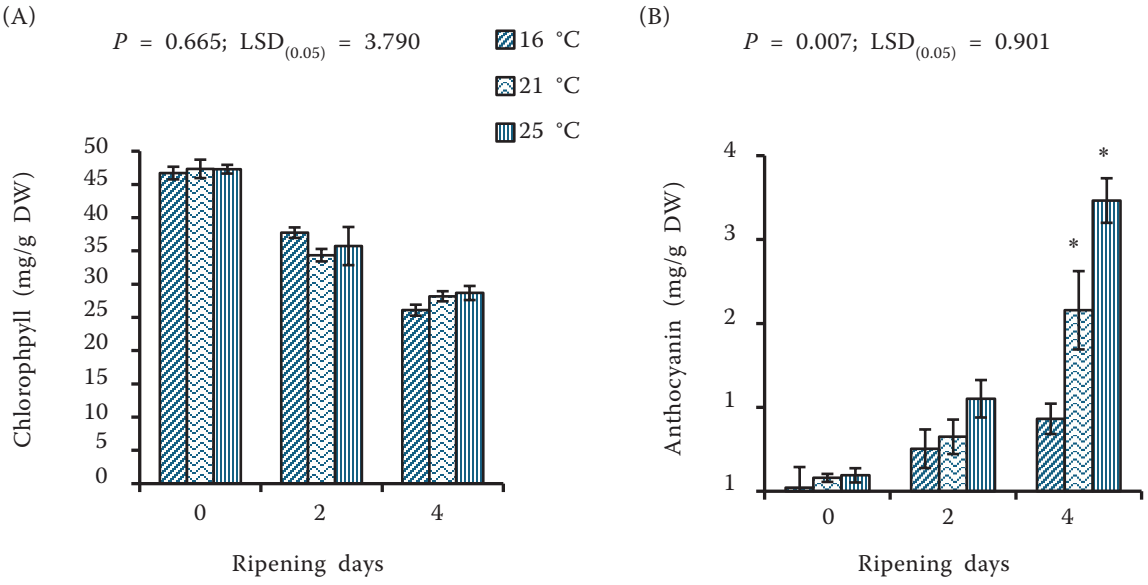

Figure 2. Changes in total chlorophyll (A) and anthocyanin content (B) of 'Hass' avocado exocarp during ripening
Fruit were stored at 5.5 °C for 28 days and ripened at 16, 21 and 25 °C. Error bars represent the standard error of 3 replicates. An asterisk (*) represents a significant difference in means according to the LSD test ($P \leq 0.05$)

https://doi.org/10.17221/72/2023-HORTSCI

it is noteworthy that Cox et al. (2004) experimented on late-season 'Hass' avocado fruit, which are not prone to colour desynchronisation (Mathaba et al. 2015; Munhuweyi et al. 2020). Contrary to our findings, an earlier report (Mathaba et al., 2015) posited that poor colouration to a lesser extent was caused by ripening temperatures in early-season 'Hass' avocado fruit, but external chilling damage associated with fruits harvested from lower slopes. However, the current study indicates that temperatures affected exocarp colouration during ripening by acting on anthocyanins. Anthocyanin concentrations were significantly higher in fruit ripened at 25 °C than in fruit ripened at 21 °C, while fruit ripened at 16 °C resulted in the lowest concentrations at the end of ripening (Figure 2B).

One potential physiological mechanism behind this observation is the influence of temperature on enzyme activity involved in anthocyanin biosynthesis. Higher temperatures (≥ 25 °C) may enhance the activity of many enzymes belonging to the flavonoid pathway (Zhang et al. 2012; Niu et al. 2017), leading to an upregulation of anthocyanin synthesis. According to Zhang et al. (2012), higher temperatures (27 °C) result in a higher accumulation of anthocyanins through upregulation of the PyMYB10 transcription gene and the phenylalanine ammonia-lyase (PAL) enzyme in 'Yunhongli No. 1' pears. In 'Red Beauty' plum fruit, it was observed that during ripening, chalcone synthase (CHS), anthocyanin synthase (ANS), dihydroflavonol 4-reductase (DFR), and PAL enzyme activities increased faster at 35 °C than at 20 °C, while UPD-glucose: flavonol 3-O-D glucosyltransferase (UFGT) activity was unaffected (Niu et al. 2017). This is supported by studies demonstrating temperature-dependent changes in activation energies for pigment-modifying enzymes in 'Hass' avocados (Gwanpua et al. 2018). Furthermore, the observed lower anthocyanin concentration in avocado fruit ripening at 16 °C could be associated with the suppression of myelo blastosis (MYB) transcription factor genes and CHS. According to Mao et al. (2022), mitogen-activated protein kinase (FvMAPK3) plays a crucial role in regulating poor fruit colouration in 'Benihoppe' strawberries in response to low temperatures. FvMAPK3 suppresses the accumulation of anthocyanin at lower temperatures (4 or 10 °C), primarily by promoting the degradation of CHS through Kelch domain-containing Fbox protein (FvKFB1) and reducing the transcriptional activity of FvMYB10. Moreover, the avocado fruit's stress response to higher temperatures may also influence anthocyanin levels. Higher temperatures (25 °C) are perceived as a form of stress, leading to increased production of abscisic acid (ABA) (Vincent et al. 2020). ABA has been identified as a crucial hormone in responding to various stresses and triggering anthocyanin production in 'Red Fuji' apples and 'Kent' mangoes (Wang et al. 2020; Kumar et al. 2020). In a comparative analysis between green-ripe and black-ripe 'Hass' avocado fruit, Arancibia-Guerra et al. (2022) discovered that black colouration was most strongly associated with total anthocyanin content, ABA levels, ACS, CHS, PAL and flavanone 3-hydroxylase expression, in descending order of importance.

Correlation analysis. Pearson correlation analysis was conducted to determine the relationship between exocarp firmness, visual colour, chromaticity and pigments (Table 2). There was a strong negative correlation between firmness and visual colour with increasing ripening temperature at 25 °C (r = –0.965), 21 °C (r = –0.944), and 16 °C (r = –0.886). The correlation between $h°$ and firmness was moderate at 16 °C (r = 0.658) compared to 21 °C (r = 0.251) or 25 °C (r = 0.513). According to the correlations, firmness loss and visual colour development are better coordinated at high ripening temperatures; however, the decline in firmness and $h°$ occur asynchronously at 21 °C compared to 16 °C and 25 °C. In this study, visual colour and $h°$ showed a strong negative correlation at 16 °C (r = –0.877), but moderate at 25 °C (r = –0.565) and 21 °C (r = –0.440). Furthermore, $h°$ was strongly correlated with total chlorophyll for fruit ripened at 16 °C (r = 0.817), and moderately at 21 °C (r = 0.666) and 25 °C (r = 0.442). However, the correlation between $h°$ and total anthocyanin was poor across all temperature regimes. Results showed a direct relationship between colour intensity ($h°$) and total chlorophyll content at 16 °C, but that relationship diminished at 21 and 25 °C. This finding corroborates those of Yang et al. (2009) who found that chlorophyll degradation occurs faster when temperatures increase within 20–30 °C in 'Cavendish' banana fruit. During ripening, total chlorophyll and anthocyanin showed a strong negative correlation when the fruit were ripened at 25 °C (r = –0.784) compared to other ripening temperatures. The correlations suggest that fruit firmness loss and chlorophyll degradation occurred concurrently with anthocyanin accumulation during ripening at 25 °C, thus colour synchronisation.

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Table 2. Pearson correlation coefficient (*r*) between firmness, visual colour, chromaticity and pigments of 'Hass' avocado fruit during ripening at 16, 21 and 25 °C

Correlation (*r*)	16 °C	21 °C	25 °C
Firmness × visual colour	-0.886^{***}	-0.944^{***}	-0.965^{***}
Firmness × L^*	0.573^{*}	0.783^{***}	0.919^{***}
Firmness × C^*	0.870^{***}	0.931^{***}	0.931^{***}
Firmness × $h°$	0.658^{**}	0.251^{ns}	0.513^{*}
Firmness × total chlorophyll	0.838^{***}	0.833^{***}	0.843^{***}
Firmness × total anthocyanin	-0.449^{ns}	-0.674^{**}	-0.892^{***}
Visual colour × L^*	-0.706^{**}	-0.838^{***}	-0.929^{***}
Visual colour × C^*	-0.948^{***}	-0.937^{***}	-0.933^{***}
Visual colour × $h°$	-0.877^{***}	-0.440^{ns}	-0.565^{*}
Visual colour × total chlorophyll	-0.950^{***}	-0.869^{***}	-0.802^{***}
Visual colour × total anthocyanin	0.599^{**}	0.598^{**}	0.810^{***}
Total chlorophyll × L^*	0.664^{**}	0.816^{***}	0.809^{***}
Total chlorophyll × C^*	0.905^{***}	0.862^{***}	0.867^{***}
Total chlorophyll × $h°$	0.817^{***}	0.666^{**}	0.442^{ns}
Total chlorophyll × total anthocyanin	-0.609^{**}	-0.570^{*}	-0.784^{***}
Total anthocyanin × L^*	-0.112^{ns}	-0.619^{**}	-0.849^{***}
Total anthocyanin × C^*	-0.412^{ns}	-0.688^{**}	-0.849^{***}
Total anthocyanin × $h°$	-0.457^{ns}	-0.081^{ns}	-0.307^{ns}

ns – no significance $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; *L* – lightness; C – chroma; $h°$ – hue angle

CONCLUSION

In conclusion, this study highlights the temperature-dependent regulation of anthocyanin content in early-season 'Hass' avocado fruit exocarp. Fruit ripened at 25 °C had higher anthocyanin accumulation with better visual colour than those ripened at 21 and 16 °C. The current study demonstrated that temperature is a critical factor for colour synchronization and that certain enzymes and phytohormones may be involved, as alluded to in the discussion. It is suggested that temperature effect on the expression of anthocyanin biosynthetic enzymes and hormones be included in future studies to validate the regulatory mechanism of colour development by temperatures in 'Hass' avocado fruit especially when the fruit is less mature, as in early-season fruit. Suitable comparisons should be done with more mature later-season fruit to help confirm the suggested mechanisms.

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Received: July 4, 2023
Accepted: October 13, 2023
Published online: May 29, 2024