#### **JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH 2021, VOL. 4(4), 439-452**



**Journal of Horticulture and Postharvest Research** 

Journal homepage: www.jhpr.birjand.ac.ir

# **Effect of postharvest glucose infusion on exocarp colour of 'Hass' avocado (***Persea americana* **Mill) during ripening**

# **Kingsly Shikwambana1\*, Tieho P. Mafeo<sup>1</sup> and Nhlanhla Mathaba<sup>2</sup>**

1, *School of Agriculture and Environmental Sciences, Faculty of Science and Agriculture, University of Limpopo, Private Bag X 1106, Sovenga, 0727, Polokwane, South Africa*

2, *Postharvest Management and Food Security, School of Agricultural Science, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Private Bag X11283, Nelspruit, 1200, South Africa*

#### **A R T I C L E I N F O A B S T R A C T**

#### **Original Article**

#### **Article history:**

Received 13 April 2021 Revised 24 July 2021

Accepted 8 August 2021

Available online 8 October 2021

**Keywords:**

Anthocyanin

Chlorophyll

Cyanidin-3-O-glucoside

*Persea americana* Mill.

**DOI: 10.22077/jhpr.2021.4254.1202** P-ISSN: 2588-4883 E-ISSN: 2588-6169

**\*Corresponding author:**

*School of Agriculture and Environmental Sciences, Faculty of Science and Agriculture, University of Limpopo, Private Bag X 1106, Sovenga, 0727, Polokwane, South Africa.*

#### **Email[: Shikwambanakingsly@gmail.com](mailto:Shikwambanakingsly@gmail.com)**

© This article is open access and licensed under the terms of the Creative Commons Attribution License <http://creativecommons.org/licenses/by/4.0/> which permits unrestricted, use, distribution and reproduction in any medium, or format for any purpose, even commercially provided the work is properly cited.

**Purpose:** The rejection of fruits because of insufficient purple colour in the exocarp has limited the profitability of 'Hass' avocado (*Persea americana* Mill) fruit. Thus, this study investigated whether glucose infusion through the pedicel can trigger anthocyanin pigment synthesis and accumulation of early harvested 'Hass' avocado exocarp, thereby resulting in improved colour development during ripening. **Research method:** 'Hass' fruit were continuously infused through the pedicel with distilled water and different glucose concentrations; 0.05, 0.13, 0.28 mM and control, therefore, assessed for fruit quality; firmness loss, visual colour) chromaticity parameters (lightness-L\*, chroma-C\* and hue-*h°*) and exocarp pigments (chlorophyll, carotenoids, anthocyanin and cyanidin-3-*O*-glucoside) during ripening at 25°C. **Findings:** Infusion of glucose and distilled water extended the ripening period by one day. Chlorophyll degradation was delayed in response to glucose infusions and distilled water compared to control. Results indicated that 0.05 and 0.13 mM glucose infusion increased anthocyanin and cyanidin-3-*O*glucoside accumulation in the exocarp, correspondingly improving exocarp colour (visual colour) after 8 days at 25°C when compared with 0.28 mM, distilled water and control. **Limitations:** Anthocyaninregulating enzymes and genes of the 'Hass' avocado fruit have not yet been identified. **Originality/Value:** This study provides insight into the possible role of  $C_6$  sugars in 'Hass' avocado fruit during postharvest ripening. By encouraging carbohydrate accumulation in the avocado fruit exocarp, postharvest poor exocarp colouration could be controlled.

**of Birjand**



## **INTRODUCTION**

Avocado (*Persea americana* Mill) 'Hass' is a popular international cultivar that has increased demand in several importing countries. Generally, the exocarp of 'Hass' avocado fruit changes from green to purple and black during ripening (Cox et al., 2004). In many cases, however, uniform purple or black is not achieved during ripening. Therefore, fruit harvested early and middle season may remain green or develop a multicoloured appearance, devaluing their commercial value and making them unattractive to consumers. Due to this conundrum, fruit rejected by consumers for not meeting quality standards results in large postharvest losses.

The colour of the exocarp of 'Hass' avocado fruit is one of the main traits producers and consumers use to determine ripeness (Mathaba et al., 2015). During ripening, chlorophyll and anthocyanin pigments are responsible for the colouration of the exocarp of 'Hass' avocado fruit (Ashton et al., 2006). Thus, during ripening, chlorophyll is degraded and anthocyanin is synthesized and accumulated. Several authors have reported that cyanidin -3-*O*-glucoside anthocyanin synthesis and accumulation confers ripe 'Hass' avocado fruit the purple and dark black exocarp colour [\(Ashton et al., 2006;](#page-11-0) [Cox et al., 2004;](#page-11-1) [Donetti & Terry, 2014\)](#page-12-0). Therefore, the poor exocarp colouration conundrum of 'Hass' avocado fruit can be controlled during ripening by triggering the pathway responsible for anthocyanin biosynthesis. Peng et al. (2016) found that the increase in anthocyanin concentration was related to the enzymatic activity responsible for the biosynthetic pathway phenylalanine ammonia-lyase and uridine diphosphate glucose flavonoid 3-*O*-glucosyltransferase.

Anthocyanin synthesis is induced by glucose by modifying cytosolic sucrose content (McKibbin et al., 2006; Xu et al., 2014). Shi et al. (2014) observed a strong correlation between anthocyanin biosynthesis and soluble sugar levels in Chinese bayberry 'Dongkui' fruit. The authors found that higher anthocyanin concentration was correlated with an increased expression of enzymes involved in the biosynthesis of anthocyanin and genes related to sugar metabolism. The phosphorylation of the uridine diphosphate (UDP) glucose molecule is required for the regulation of genes encoding anthocyanin biosynthesis (Chen et al., 2013; Wang et al., 2019). Due to the importance of UDP-glucose as a precursor in secondary metabolites and subsequently, anthocyanin biosynthesis, we investigated whether postharvest treatment with glucose can improve avocado fruit colour by triggering the flavonoid pathway.

The avocado fruit consists of seed, mesocarp and exocarp (which contain a proportion of carbohydrates). Tesfay et al.  $(2012)$  and Tesfay et al.  $(2009)$  reported on the function of carbohydrates in 'Hass' avocado. According to the authors, carbohydrate carbon seven  $(C_7)$ dominates the mesocarp at different stages of an avocado life cycle and serves as an energy source and antioxidant. The  $C_7$  sugar in avocado fruit 'Hass' was reported to be the main energy source after harvest. To date,  $C_7$  sugars are unknown to play any role in exocarp colour development of 'Hass' avocados. There is a possibility that carbon-six  $(C_6)$ carbohydrates are also involved. Hence, it is reasonable to investigate the role of  $C_6$  sugar in avocado during ripening. Hu et al. (2016) report that sucrose, glucose, and fructose are critical for anthocyanin synthesis. It was found that an exogenous supply of sugar and sucrose triggered anthocyanin synthesis in isolated leaves, leaf disks, and suspension cultures (Larronde et al., 1998). Weiss (2000) reported that sugars, light and plant hormones interact to induce anthocyanin biosynthesis and expression of structural genes in 'Petunia hybrid' corollas.



In *Arabidopsis thaliana* flower and 'Orin' apple fruit, sucrose and biosynthesis pathway for uridine diphosphate glucose (UDP-gluc) contributed to the biosynthesis of cyanidin -3 galactoside anthocyanin pigment [\(Ban et al., 2009;](#page-11-2) [Sivitz et al., 2008\)](#page-12-1). This study aimed to investigate whether glucose infused through fruit pedicel could increase anthocyanin synthesis and accumulation in the exocarp of early harvested 'Hass' avocados, resulting in improved colour development during ripening.

# **MATERIALS AND METHODS**

### **Equipment and chemical reagent**

The experiment was conducted in the Postharvest Laboratory, Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC) and Chemistry Laboratory, Tshwane University of Technology. All chemicals used for sample preparation, extraction and mobile phases were methanol, acetonitrile, acetone, acetic acid, potassium chloride, hydrochloric acid, sodium acetate and the standard was cyanidin -3-*O*-glucoside chloride from Sigma-Aldrich chemical, Merck company, South Africa.

# **Collection and treatment of 'Hass' avocado fruits**

In April 2019, early matured 'Hass' avocado fruit were harvested with 10 cm pedicle at commercial standard using dry matter (21 % DM) maturity from Nico Swart Trust commercial farm at Kieparsol (Hazyview, Mpumalanga, South Africa, GPS: 25°29'19'' S; 31°13'67'' E). Harvested 'Hass' with 10 cm pedicel were carefully placed in crates and immediately transported to the Agricultural Research Council - Tropical and Subtropical Postharvest Laboratory in Nelspruit (Mpumalanga, South Africa, GPS: 25°45'18''S; 30°96'97'' E). Upon arrival at the laboratory, 'Hass' avocado fruit pedicles were re-cut to 5 cm length, and 15 mL tubes were interleaved on the apex of the fruit with 5 cm pedicles inside the tube and at the bottom (where the apex of the fruit and silicon tube meet) as previously described by Bertling & Tesfay (2011), bostik prestik and petroleum jelly were applied to prevent leakages. Five treatments were: control fruit with pedicel, and infused with distilled water and glucose concentrations [in millimole (mM)]: 0.05, 0.13 and 0.28 mM. Ninety (90) fruit per treatment were continuously infused with 10 mL/fruit of the above treatments. Controls were also included in which fruit with pedicel were not treated with any solution. Thereafter, distilled water, glucose infused, and control fruit were stored at 5.5 °C for up to 28 days. After cold storage, the treated and control fruit were kept at ambient temperature 25±2°C for ripening. The experiment was conducted as a completely randomized design (CRD) with three replications per treatment.

#### **Fruit quality analysis**

# *Firmness measurement*

Fruit firmness was determined using an automated Sinclair  $IQ^{TM}$  desktop machine (51DFTB, International Ltd, Jorrold, Bowthorpa, Norwich, NR5, 9.D, England). Fruit were measured three times along the equatorial region and their firmness was calculated in Newtons (N). The same fruit were evaluated continuously.

# *Colour measurement*

As shown in plate 1, avocado 'Hass' exocarp colour change was determined subjectively using a visual colour rating scale. A Minolta Chromameter calibrated to CIE L\*, a\*, and b\* coordinates (Model CR-400, Minolta Corp., Osaka, Japan) was used to determine the



objective colour parameters. The chroma  $(C^*)$  and hue angle  $(h^{\circ})$  were calculated using McGuire's formulas (1992).

Chroma (C \*) = 
$$
\sqrt{(a * )^2 + (b * )^2}
$$
 (1)  
Hue angle (h°) =  $\theta$  + arctan  $\left(\frac{b^*}{a^*}\right)$  (2)



**Plate 1.** Visual colour rating scale (1= emerald green,  $2 =$  forest green,  $3 =$  olive green,  $4 =$  violet,  $5 =$  purple, and  $6 = black$ ) of 'Hass' avocado fruit during ripening.

# *Fruit sampling and peel preparation*

During the evaluation period (0, 2, 4, 6 and 8 days), exocarps of five fruits were manually separated and dried using a freeze dryer. The dried exocarp samples were ground into fine powder using liquid nitrogen and a pistol and mortar. As soon as the fine powder was formed, it was stored at -80°C in clean vials until needed

# *Total chlorophyll and total carotenoids measurement*

Using the method of Lichtenthaler (1987) with a few modifications, the concentrations of chlorophyll and carotenoids were determined. The sample of 0.5 grams of finely powdered avocado exocarp was mixed with 10 mL of 80% (v/v) acetone for 30 minutes on ice. A spectrophotometer (UV-1800, Shimadzu, Corp. Japan) was used to read the supernatants at 663 nm, 646 nm and 470 nm after centrifugation for 5 minutes at 6000  $\times$  g. The following equations were used to calculate the total chlorophyll and carotenoids.



Where C*a* referred to chlorophyllide *a* and C*b* referred to chlorophyllide *b*

# *Total anthocyanin and cyanidin -3-O-glucoside concentration*

Extractions were conducted as described by Cox et al. (2004) with few modifications. 0.5 grams of fine avocado peel powder were mixed with 5 mL of 10 % acetic acid/methanol  $(v/v)$ at room temperature, centrifuged for 10 minutes at  $3000 \times g$ , and diluted with methanol: water: acetic acid (50:50:10,  $v/v/v$ ). This study used the pH differential method previously described by Giusti and Wrolstad (2001) to determine anthocyanin concentration. To filter the 1:1 diluted supernatant, 0.45 mm nylon filters were used. A mixed buffer of potassium chloride buffer (pH1.0) and sodium acetate buffer (pH4.5) was added to the filtrate. A spectrophotometer (UV-1800, Shimadzu, Cor. Japan) was used to measure the absorbance at 530 and 700 nm after 10 minutes of incubation in the dark. Formulas (7) and (8) were used to calculate the total anthocyanin. Analyzing the concentration of cyanidin-3-O-glucoside was performed using an HPLC equipped with JASCO units (LG-980-02 ternary gradient



controller, AS-950 autosampler, and UV-975 UV/Vis detector). Column used was a Phenomenex AQUA 5u C18 125A 5 um PR-18e 4.6 ×150 mm (Los Angeles, USA), kept at 35 °C. The mobile phases were: (A) 1.5 % H3PO4 and (B) acetic acid: acetonitrile: H3PO4: water (20: 24: 1.5: 54.5,  $v/v/v/v$ ). In the solvent program, solvent (B) began at 20 %, increased to 70 % after 25 minutes, then 90 % after 30 minutes. After 35 minutes, the solvent composition was returned to the initial 20 % solvent (B) and was ready for the next injection. The volume of sample injection was 2 l and the wavelength of detection was 530 nm.

> $A = (A530 - A700)pH1.0 - (A530 - A700)pH4.5$ (7) Total anthocyanin =  $(A \times MW \times DF)/(E \times L)$ (8)

Where A= Absorbance,  $\epsilon$  = cyanidin -3-glucoside molar absorbance (26900), MW = anthocyanin 164 molecular weight (449.2),  $\overline{Df}$  = dilution factor, L = cell path length (1 cm)

#### **Statistical analysis**

The analysis of variance (ANOVA) was performed using GenStat  $16<sup>th</sup>$  edition (VSN International, UK). The Least Significant Difference (LSD) was used to calculate the p-value at a confidence interval of 95 %. Pearson's correlation was used to examine the relationship between pericarp pigments (chlorophyll, carotenoid, anthocyanin, and cyanidin -3-*O*glucoside) and firmness (L\*, C\* and *h*°).

# **RESULTS AND DISCUSSION**

#### **Fruit firmness**

In 'Hass' avocado fruit, firmness is the main indicator of eating quality (Ahmad et al., 2013). All treatments resulted in a decrease in firmness at  $25^{\circ}$ C (Fig. 1). A decrease in fruit firmness correlated negatively and significantly with an increase in exocarp colour  $(r = -0.85)$  while chromaticity colour parameters were positively correlated;  $L^*$  ( $r = 0.89$ ),  $C^*$  ( $r = 0.94$ ) and  $h^{\circ}$  $(r = 0.81)$  (Table 1), consistent with [Cox et al. \(2004\)](#page-11-1). Figure 1 shows that the control fruit ripened rapidly after cold storage and became fully ripe within 6 days. Infusion of glucose and distilled water, however, extended the ripening period by one day. The results were in agreement with Bertling and Tesfay  $(2011)$ , showing that infusion of carbon-seven  $(C_7)$  sugar significantly enhanced the shelf-life of the 'Hass' avocado mesocarp by maintaining Dmannoheptulose and perseitol sugar pools. A study by Mathe  $(2018)$  also showed that  $C_7$  (Dmannoheptulose and perseitol) and sucrose increased fruit firmness, fresh mass retention, and respiration rate. In both glucose and distilled water infused fruit, the firmness was greater than that of control fruit (Fig. 1). Although no differences were noticed after 6 and 8 days, fruit infused with 0.13 mM and distilled water maintained higher firmness than fruit infused with 0.05 mM and 0.28 mM. Results obtained here are in line with previous studies on sugar and water infused into avocado fruit (Bertling & Tesfay, 2011; Blakey et al., 2009; Mathe, 2018). These studies have shown that infusing sugars such as D-mannoheptulose, perseitol, and sucrose to 'Fuerte' and 'Hass' avocados, as well as water, resulted in a decreased rate of respiration, increased firmness, and extended shelf-life. In this study, glucose and distilled water infusions improved water balance and maintained the fruit's turgidity, extending shelf life. Bertling and Tesfay (2011) found that D-mannoheptulose and water infused into fruit resulted in firmer fruit than the control fruit. It is likely that glucose infusion resulted in increased water potential in the fruit, which reduced ethylene production, causing the fruit to ripen more slowly than control fruit.





**Fig. 1.** Effect of distilled water and glucose infusion through pedicel on the firmness of 'Hass' avocado fruit during ripening. Values are means of 3 replicates of 30 fruits. Error bars indicate  $\pm$ SE of means at  $p \le 0.05$ .

#### **Fruit exocarp colour**

Colour change as measured by visual colour rating significantly ( $p = 0.012$ , Fig. 2a) increased for all treatments at 25°C. As shown in Figure 2a, the visual colour intensity increased slightly for the treatments during the first 4 days at 25°C but increased sharply after ripening (6 days for control) and 8 days for the infused treatments. However, 'Hass' avocado fruit infused with glucose showed improved exocarp colour development when compared with control and distilled water as evident from day 4 until day 8. After 6 days at 25°C, the control fruit developed to olive green (visual colour  $= 3.42$ ) when compared with other treatments. After day 8 at 25°C, the visual colour of fruit infused with glucose at 0.05, 0.13 and 0.28 mM developed purple colour (visual colour  $= 5.25$ ), violet (visual colour  $= 4.92$  and 4.42), respectively (Plate 1 and 2). Whereas, fruit infused with distilled water only showed traces of olive-green colour (visual colour = 3.92) on day 8 at 25 $^{\circ}$ C. This study found that the visual colour correlated positively with anthocyanin content  $(r = 0.84)$ , suggesting that glucose infusion contributed to colour change. Bolouri-Moghaddam et al. (2010) demonstrated that glucose acts as a signal molecule and is involved in anthocyanin synthesis.

Moreover, the values of chromaticity parameters  $(L^*, C^*$  and  $h^{\circ})$  decreased with time to ripening for all treatments during the colour development and ripening (Fig. 2a-d). Cox et al. (2004) and Donetti and Terry (2014) reported that avocado 'Hass' decreased its L\*, C\*, and *h*° values. Control fruit measured a lower C\* value and higher *h*° value after 6 days when compared to glucose and distilled water infused fruit. Compared with distilled water, all glucose infused fruit showed lower L\* and *h*° values after 6 and 8 days. An indication that glucose plays a positive role in the development of avocado exocarp colour could be found here. The chromaticity parameters  $(L^*, C^*,$  and  $h^{\circ})$  also correlated with total chlorophyll ( $r =$ 0.72, 0.76, and 0.68, respectively) and anthocyanin ( $r = -0.73$ ,  $-0.67$  and  $-0.73$ , respectively, Table 1), possibly due to glucose treatment. Consequently, anthocyanins were significantly and negatively correlated with chlorophylls. Sugar has been shown to enhance the accumulation of anthocyanin pigments in fruit crops; therefore, it may contribute to the colouration of exocarps (Das et al., 2012; Huang et al., 2019). According to [Xu et al.](#page-13-0) (2014) and [Yang et al.](#page-13-1) (2013), hexokinase (HXK1) phosphorylates glucose to produce uridine diphosphate glucose (UDP), an essential precursor of anthocyanin and cyanidin-3- Oglucoside biosynthesis.





**Fig. 2.** Effect of distilled water and glucose infusion through pedicel on subjective (visual colour) (**a**) and chromaticity parameters (L\*, C\* and *h°*, **b, c** and **d**, respectively) of 'Hass' avocado fruit during ripening. Values are means of 3 replicates of 30 fruits. Error bars indicate  $\pm$ SE of means at p $\leq$  0.05.



# **Chlorophyll and carotenoids content**

All treatments showed significant declines in total chlorophyll ( $p = 0.002$ ), chlorophyll-a ( $p =$ 0.002), chlorophyll-b ( $p = 0.004$ ), and total carotenoids ( $p = 0.002$ ) (Fig. 3a-d). These results agree with Ashton et al. (2006). Color assessment was negatively correlated with total chlorophyll ( $r = -0.68$ ), as well as total carotenoids ( $r = -0.71$ ) (Table 1). However, control fruit showed a slower decrease in total chlorophyll, chlorophyll a, chlorophyll b, and total carotenoids from day 0 to 2 at 25°C, but the content gradually decreased after 4 days, reaching somewhat lower levels at day 6. Chlorophyll degradation was delayed in response to glucose infusions and distilled water compared to control. The chlorophyll and carotenoids deteriorated more slowly in the presence of 0.05 mM than those infused with 0.13 and 0.28 mM. The reason for this may be due to the high water potential from infusion treatment, which led to a reduction in abscisic acid (ABA) and ethylene production (Blakey, 2011). In the study by Zaharah et al.  $(2013)$ , it was demonstrated that ABA enhances ethylene biosynthesis, as shown by its effects on fruit ripening. Infusing glucose and distilled water delayed the biosynthesis of ethylene by reducing ABA levels. Lower ABA levels delayed ethylene biosynthesis, resulting in delayed degradation of chlorophyll and carotenoid.

# **Total anthocyanin and cyanidin -3-***O***-glucoside concentration**

The main anthocyanin responsible for the purple and black colour during ripening in 'Hass' avocados are cyanidin-3-O-glucoside (Cox et al., 2004). Our study showed that total anthocyanin concentration increased significantly  $(p< 0.001)$  for all treatments during ripening. Compared with all other treatments, fruit infused with 0.13 mM had higher total anthocyanin concentrations. The anthocyanin concentration was higher with glucose-infused fruit after 8 days at 25°C than with control fruit or distilled water (Fig. 4a). A significant and positive correlation was found between total anthocyanin and cyanidin-3-O-glucoside  $(r =$ 0.84), corroborating previous findings (Cox et al., 2004; Donetti & Terry, 2014).

Cyanidin-3-O-glucoside concentrations increased significantly as ripening time increased among treatments. In fruit infused with 0.05, 0.13 mM glucose and distilled water, the concentration of cyanidin-3-O-glucoside increased after 2 to 8 days at 25°C, however, glucose was higher than distilled water (Fig. 4b). These results agree with several other studies that have demonstrated that exogenous sugars can induce anthocyanin accumulation in various plants (Ai et al., 2016; Hu et al., 2016). Glycosylation of uridine diphosphate glucose (UDPgluc) is required for the formation of anthocyanin. The increase in cyanidin-3-*O*-glucoside concentration after glucose infusion can be attributed to the provision of UDP-glucose. According to Das et al. (2012), glucose, fructose, and sucrose alter gene expression involved in anthocyanin synthesis and accumulation, with signal transduction pathways as well. Huang et al. (2016) reported that glucose regulated MdHXK1 and MdbHLH3 anthocyanin biosynthesis in 'Red Delicious' apple fruit. In the 'Mirage rose' Petunia hybrid flower, sucrose enhanced the anthocyanin content by increasing transcription factors involved in the induction of genes involved in anthocyanin biosynthesis, according to Ai et al. (2016). In the current study, glucose was infused into 'Hass' avocado fruit during ripening, but its effects on anthocyanin biosynthesis were not clarified.









**Fig. 3.** Effect of distilled water and glucose infusion through pedicel on total chlorophyll (**a**), chlorophyll-a (**b**), chlorophyll-b (**c**) and total carotenoids (**d**) of 'Hass' avocado fruit during ripening. Values are means of 5 fruits. Error bars indicate  $\pm$ SE of means at p $\leq$  0.05.



**Fig. 4.** Effect of distilled water and glucose infusion through pedicel on total anthocyanin (**a**) and cyanidin -3-*O*glucoside concentration (**b**) of 'Hass' avocado fruit during ripening. Values are means of 5 fruits. Error bars indicate  $\pm$ SE of means at p $\leq$  0.05.

**Table 1.** Correlation coefficients among the evaluated quality attributes

	$L^*$	Visual colour	~ C*	$h^{\circ}$	$\overline{\phantom{a}}$ Total anth	Cy3Glu	Chl-a	$Chl-b$	<b>Total Chl</b>	Total caret
Firmness	$0.89**$	$-0.85**$	$0.94**$	$0.81**$	$-0.60**$	$-0.63**$	$0.80**$	$0.77**$	$0.80**$	$0.81**$
$L^*$		$-0.93**$	$0.97**$	$0.89**$	$-0.73**$	$-0.80**$	$0.71**$	$0.71**$	$0.72**$	$0.78**$
visual			$-0.89**$	$-0.95**$	$0.84**$	$0.87**$	$-0.67**$	$-0.68**$	$-0.68**$	$-0.71**$
colour										
$C^*$				$0.85**$	$-0.67**$	$-073**$	$0.76**$	$0.74**$	$0.76***$	$0.80**$
$h^{\circ}$					$-0.73**$	$-0.79**$	$0.61**$	$0.65**$	$0.63**$	$0.67**$
Total ant						$0.84**$	$-0.55**$	$-0.57**$	$-0.56**$	$-0.59**$
Cy3Glu							$-0.44**$	$-0.44**$	$-0.45**$	$-0.51**$
Chl-a								$0.94**$	$0.99**$	$0.81**$
$Chl-b$									$0.97**$	$0.74**$
<b>Total Chl</b>										$0.79**$

 $L^*$  = Lightness,  $C^*$  = Chroma,  $h^{\circ}$  = hue angle, Total ant = Total anthocyanin, Cy3Glu = Cyanidin -3-O-glucoside, Chl-a = Chlorophyll a,Chl-b = Chlorophyll b, Total Chl = Total chlorophyll, Total caret = Total carotenoids,  $* = p < 0.05$  and  $** = p < 0.01$ 

#### **Correlation analysis**

Table 1 shows the Pearson correlation coefficients between firmness, colour attributes, and exocarp pigments. Firmness was strongly correlated with visual color  $(r = -0.85)$ , anthocyanin total ( $r = -0.60$ ) and cyanidin  $-3$ -O-glucoside ( $r = -0.63$ ). This suggests that the 'Hass' avocado fruit became softer and darker as they ripened. There was a significant negative correlation between  $h^{\circ}$  values and visual color ( $r = -0.95$ ), total anthocyanins ( $r = -0.73$ ), and cyanidin-3-O-glucosides ( $r = -0.79$ ). It has been reported that changes in exocarp colour are related to cyanidin-3-O-glucoside content (Cox et al., 2004; Ashton et al., 2006, Donetti and Terry, 2011). Total chlorophyll and chlorophyll a  $(r = 0.99)$  and chlorophyll b  $(r = 0.97)$  were positively correlated. According to Ashton et al (2006), 'Hass' avocado fruit has higher chlorophyll levels due to their high chlorophyll a and chlorophyll b content. There is a negative correlation between chlorophyll content and total anthocyanin  $(r = -0.59)$  and



cyanidin -3-O-glucoside concentration  $(r = 0.51)$ . According to Cox et al. (2004), the development of the exocarp colour is caused by chlorophyll breakdown and anthocyanin accumulation.



Ripening time (Days at 25 °C)

**Plate 2.** Effect of distilled water and glucose infusion through pedicel on exocarp colour development of 'Hass' avocado fruit during ripening**.**



# **CONCLUSION**

Avocado 'Hass' exocarp develops poor colour due to an insufficient accumulation of cyanidin -3-O-glucoside during ripening. The result in this study showed that continuous glucose infusion at 0.05 and 0.13 mM through the fruit pedicel resulted in an increased total anthocyanin and cyanidin -3-*O*-glucoside concentration, concomitantly, the fruit developed to the purple (visual colour  $= 5$ ) exocarp colour after 8 days at 25 $\degree$ C. For control of postharvest poor exocarp colouration in 'Hass' avocados, production practices that increase sugar accumulation in the exocarp may prove effective.

#### **Acknowledgement**

The authors are grateful to the National Research Foundation (NRF) for their financial support. The Agricultural Research Council – Tropical and Subtropical Crops (ARC-TSC) postharvest division for financial and technical support.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

#### **REFERENCES**

- Ahmad, A., Ali, Z.M., & Zainal, Z. (2013). Effect of 1-methylcyclopropene (1-MCP) treatment on firmness and softening related enzymes of'sekaki'papaya fruit during ripening at ambient. *Sains Malaysiana, 42*, 903-909.
- Ai, T.N., Naing, A.H., Arun, M., Lim, S.H. & Kim, C.K. (2016). Sucrose-induced anthocyanin accumulation in vegetative tissue of Petunia plants requires anthocyanin regulatory transcription factors. *Plant Science 252*, 144-150. https://doi.org/10.1016/j.plantsci.2016.06.021
- <span id="page-11-0"></span>Ashton, O.B., Wong, M., McGhie, T.K., Vather, R., Wang, Y., Requejo-Jackman, C., Ramankutty, P. & Woolf, A.B. (2006). Pigments in avocado tissue and oil. *Journal of Agricultural and Food Chemistry, 54*(26), 10151-10158. https://doi.org/10.1021/jf061809j
- <span id="page-11-2"></span>Ban, Y., Kondo, S., Ubi, B.E., Honda, C., Bessho, H. & Moriguchi, T. (2009). UDP-sugar biosynthetic pathway: contribution to cyanidin 3-galactoside biosynthesis in apple skin. *Planta 230*(5), 871-881. https://doi.org/10.1007/s00425-009-0993-4
- Bertling, I. & Tesfay, S. (2011). Avocado sugars–key to postharvest shelf life? Unpublished thesis, University of KwaZulu-Natal.
- Blakey, R.J. (2011). Management of avocado postharvest physiology. PhD thesis, University of KwaZulu-Natal.
- Blakey, R.J., Bower, J.P. & Bertling, I. (2009). Influence of water and ABA supply on the ripening pattern of avocado (*Persea americana* Mill.) fruit and the prediction of water content using Near Infrared Spectroscopy. *Postharvest Biology and Technology 53*(1-2), 72-76. https://doi.org/10.1016/j.postharvbio.2009.03.004
- Bolouri‐Moghaddam, M.R., Le Roy, K., Xiang, L., Rolland, F. & Van den Ende, W. (2010). Sugar signalling and antioxidant network connections in plant cells. *The FEBS Journal 277*(9), 2022- 2037. https://doi.org/10.1111/j.1742-4658.2010.07633.x
- Chen, R., Song, S., Li, X., Liu, H. & Huang, D. (2013). Phosphorus deficiency restricts plant growth but induces pigment formation in the flower stalk of Chinese kale. *Horticulture, Environment, and Biotechnology 54*(3), 243-248. https://doi.org/10.1007/s13580-013-0018-x
- <span id="page-11-1"></span>Cox, K.A., McGhie, T.K., White, A. & Woolf, A.B. (2004). Skin colour and pigment changes during ripening of 'Hass' avocado fruit. *Postharvest Biology and Technology, 31*(3), 287-294. https://doi.org/10.1016/j.postharvbio.2003.09.008
- Das, P.K., Shin, D.H., Choi, S.B. & Park, Y.I. (2012). Sugar-hormone cross-talk in anthocyanin biosynthesis. *Molecules and Cells 34*(6), 501-507. https://doi.org/10.1007/s10059-012-0151-x
- <span id="page-12-0"></span>Donetti, M. & Terry, L.A. (2014). Biochemical markers defining growing area and ripening stage of imported avocado fruit cv. Hass. *Journal of Food Composition and Analysis, 34*(1), 90-98. <https://doi.org/10.1016/j.jfca.2013.11.011>
- Giusti, M.M. & Wrolstad, R.E., 2001. Characterization and measurement of anthocyanins by UV‐visible spectroscopy. *Current Protocols in Food Analytical Chemistry, 1*, (F1. 2.1-F1. 2.13. http://dx.doi.org/10.1002/0471142913.faf0102s00.
- Hu, D.G., Sun, C.H., Zhang, Q.Y., An, J.P., You, C.X. & Hao, Y.J. (2016). Glucose sensor MdHXK1 phosphorylates and stabilizes MdbHLH3 to promote anthocyanin biosynthesis in apple. *PLoS Genetics, 12*(8), e1006273. https://doi.org/10.1371/journal.pgen.1006273
- Huang, Z., Wang, Q., Xia, L., Hui, J., Li, J., Feng, Y. & Chen, Y. (2019). Preliminarily exploring of the association between sugars and anthocyanin accumulation in apricot fruit during ripening. *Scientia Horticulturae, 248*, 112-117.<https://doi.org/10.1016/j.scienta.2019.01.012>
- Larronde, F., Krisa, S., Decendit, A., Cheze, C., Deffieux, G. & Mérillon, J.M. (1998). Regulation of polyphenol production in Vitis vinifera cell suspension cultures by sugars. *Plant Cell Reports,* (17), 946-950.<https://doi.org/10.1007/s002990050515>
- Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, Methods in enzymology. *Elsevier*, 350-382. https://doi.org/10.1016/0076-6879(87)48036-1
- Mathaba, N., Mafeo, T.P. & Kruger, F.J. (2015). The skin colouring problem of 'Hass' avocado fruit during ripening. *South African Avocado Growers Association Yearbook 38*, 51-57.
- Mathe, S. (2018). Maintenance of carbon 7 sugar levels and effect on ripening of 'fuerte' and 'hass' avocado (*Persea americana* Mill.) fruit.
- McGuire, R.G. (1992). Reporting of objective color measurements. *HortScience, 27*(12), 1254-1255. https://doi.org/10.21273/hortsci.27.12.1254
- McKibbin, R.S., Muttucumaru, N., Paul, M.J., Powers, S.J., Burrell, M.M., Coates, S., Purcell, P.C., Tiessen, A., Geigenberger, P. & Halford, N.G. (2006). Production of high‐starch, low‐glucose potatoes through over‐expression of the metabolic regulator SnRK1. *Plant Biotechnology Journal, 4*(4), 409-418.<https://doi.org/10.1111/j.1467-7652.2006.00190.x>
- Peng, H., Yang, T., Whitaker, B. D., Shangguan, L., & Fang, J. (2016). Calcium/calmodulin alleviates substrate inhibition in a strawberry UDP-glucosyltransferase involved in fruit anthocyanin biosynthesis. *BMC Plant Biology*, *16*(1), 1-11[.](http://dx.doi.org/10.1186/s12870-016-0888-z) [http://dx.doi.org/10.1186/s12870-016-0888-z.](http://dx.doi.org/10.1186/s12870-016-0888-z)
- Shi, L., Cao, S., Shao, J., Chen, W., Zheng, Y., Jiang, Y., & Yang, Z. (2014). Relationship between sucrose metabolism and anthocyanin biosynthesis during ripening in Chinese bayberry fruit. *Journal of Agricultural and Food Chemistry*, *62*(43), 10522-10528. http://dx.doi.org/10.1021/jf503317k.
- <span id="page-12-1"></span>Sivitz, A.B., Reinders, A. & Ward, J.M. (2008). Arabidopsis sucrose transporter AtSUC1 is important for pollen germination and sucrose-induced anthocyanin accumulation. *Plant Physiology, 147*(1), 92-100.<https://doi.org/10.1104/pp.108.118992>
- Tesfay, S., Bertling, I. & Bower, J. (2009). Seasonal trends of specific phenols in 'Hass' avocado tissues. *I All Africa Horticultural Congress, 911,* 349-354.
- Tesfay, S., Bertling, I., Bower, J. & Lovatt, C. (2012). The quest for the function of 'Hass' avocado carbohydrates: clues from fruit and seed development as well as seed germination. *Australian Journal of Botany, 60*(1), 79-86. https://doi.org/10.1071/bt11166
- Wang, S., Wu, H., Xu, W., Ma, X., Li, L. & Zhan, R. (2019). Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of mango (Mangifera indica Linn.), IOP Conference Series: Materials Science and Engineering. IOP Publishing, (569), 022036. https://doi.org/10.1088/1757-899x/569/2/022036
- Weiss, D. (2000). Regulation of flower pigmentation and growth: multiple signaling pathways control anthocyanin synthesis in expanding petals. *Physiologia Plantarum, 110*(2), 152-157. https://doi.org/10.1034/j.1399-3054.2000.110202.x
- <span id="page-13-0"></span>Xu, W., Peng, H., Yang, T., Whitaker, B., Huang, L., Sun, J. & Chen, P. (2014). Effect of calcium on strawberry fruit flavonoid pathway gene expression and anthocyanin accumulation. *Plant Physiology and Biochemistry, 82*, 289-298. https://doi.org/10.1016/j.plaphy.2014.06.015
- <span id="page-13-1"></span>Yang, Z., Wang, T., Wang, H., Huang, X., Qin, Y. & Hu, G. (2013). Patterns of enzyme activities and gene expressions in sucrose metabolism in relation to sugar accumulation and composition in the aril of Litchi Chinensis Sonn. *Journal of Plant Physiology, 170*(8), 731-740. https://doi.org/10.1016/j.jplph.2012.12.021
- Zaharah, S. S., Singh, Z., Symons, G. M., & Reid, J. B. (2013). Mode of action of abscisic acid in triggering ethylene biosynthesis and softening during ripening in mango fruit. *Postharvest Biology and Technology*, (75), 37-44. https://doi.org/10.1016/j.postharvbio.2012.07.009