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AVG Treatment at Pre-harvest to Delay Ripeness on Booth7 Avocado Based on The Activity of ACC Synthases and ACC Oxidase Enzymes

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ABSTRACT

Purpose: *The Booth7 Avocado (Persea americana Mill.) is an unusual fruit, and one of the most rapidly ripening at about 5 to 7 days after harvest. The ripening period for climacteric fruit is triggered by ethylene production; changes in ethylene intensity appear together with events of other changes in the fruit, including changes in pigment and sugar content. Hence, delaying the ripeness of Booth7 Avocado is essential in the preservation.*

Research Method: *Our study used aminoethoxyvinylglycine (AVG) treatments with 200 μL L^{-1}, 400 μL L^{-1}, and 600 μL L^{-1} to assess the impacts of AVG on Booth7 Avocado through respiratory rate, 1-amino-cyclopropane-1-carboxylic acid production, 1-aminocyclopropane-1-carboxylate oxidase (ACO) activity, and ethylene content, mass loss and spoilage ratio measured on 3-days cycle repeats of day of storage (DAS) until the fruit spoilage was not over 20%.*

Findings: *Booth7 Avocado of the control treatment discovered a novel notice by the activity of Acetyl-CoA synthetase enzymes was recorded not concurrent with the activity of the ACO enzyme. The optimal significant inhibition of AVG (400 μL L^{-1}) was determined, in which both ACC and ethylene biosynthesis was delayed to six days. The mass loss was the lowest, and the spoilage ratio was 16.67% on the 30th DAS, while the spoilage ratio in the control treatment was overloaded by 20% on the 24th DAS.*

Originality/Value: *This study revealed that AVG treatment of the content 400 μL L^{-1} could effectively delay ripeness in Booth7 Avocados.*

Keywords: *Aminoethoxyvinylglycine (AVG), ACC synthases enzyme, ACC oxidase (ACO) enzyme, Ethylene production, Ripeness*

INTRODUCTION

The Booth7 Avocado (*Persea americana* Mill.) is an unusual fruit, and one of the most rapidly ripening at about 5 to 7 days after harvest (Seymour, 1993). Today, it is cultured commonly in provinces of the central highlands and southeast region of Vietnam, especially the Tay Nguyen province, because the quality of its fruit pulp is slightly better than other cultivars (Cuong, 2002; Le, 2001). It is a rich source of monounsaturated and polyunsaturated fatty acids and contains various essential components, such as vitamins A, B, and C, and minerals, fibers, and antioxidants (Maguire, 2014). Hence, the Booth7 Avocado is considered a fruit of high economic importance for Vietnam and international trade.

The ripening period for climacteric fruit is triggered by ethylene production; changes in ethylene intensity appear together with events of other changes in the fruit, including changes in pigment and sugar content (Gambetta *et al.*, 2010; Sitbon *et al.*, 2011).

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Primarily, ethylene production in plant tissues is regulated by the activities of two key enzymes: ACC synthases and ACC oxidase (ACO) enzymes. Beginning with methionine metabolism in all fruits, S-adenosyl-L-methionine is converted to a 1-amino-cyclopropane-1-carboxylic acid (ACC) precursor by the ACS enzyme, and then ACC to produce ethylene hormone under subsequent oxidation of the ACO enzyme (Fig. 01).

Because of the significant loss of fruit due to ethylene's effects on ripening and senescence, attempts to discover potential chemical inhibitors have been made to prevent or delay fruit ripening reversibly. If aminoethoxyvinylglycine (AVG) is considered an inhibitor of ACS involved in ethylene biosynthesis, 1-methylcyclopropene (1-MCP) is known as an inhibitor of the ethylene receptor. Thus, both the above inhibitors interfere independently with two different receptors. The application of AVG and 1-MCP inhibitors on ethylene production and fruit quality has been evaluated in recent studies (Avtar *et al.*, 2012; Schaller *et al.*, 2017; Munoz-Robredo *et al.*, 2012; Sitbon *et al.*, 2011). For example, in 2005, a study on the effect of 1-MCP on stored Hass avocados (*Persea americana* Mill.) for 4 or 7 weeks at 5.5°C following treatment with 50 – 1000 nl L^{-1} 1-MCP for 6–24 hours, at 6°C or 15°C, were examined. The results of applications of lower 1-MCP concentrations and shorter treatment have caused minimal fruit ripening, but those have not impacted fruit quality. More significantly, following experimental results derived from the non-1-MCP-treated Hass avocado fruits involved in disorders responding to fruit maturity demonstrated insignificant impacts on the collected avocado firmness at pre-harvest or post-harvest. (Allan *et al.*, 2005). For Booth7 Avocado, an alternative treatment method using 1-MCP was researched with two pre-climacteric stages (Marcio *et al.*, 2014). The observed results from the collected samples in October (early season) and in November (late season) revealed that the application of 1-MCP at higher concentrations in either aqueous (2.77 mol L^{-1}) or gaseous (6.31 nmol L^{-1}) form had the most significant effects on selected pulp textural parameters and then effectively delayed ripening for early-season fruit longer than 4 days in late-season fruit.

Until now, the relationship between each inhibitor and ethylene is controversial because the degree of interference of each pathway in fruit ripening may differ according to species, stage of development, and experimental procedure (Bingxue *et al.*, 2019; Lorenzo *et al.*, 2018; Soeno *et al.*, 2010). On the other hand, the AVG inhibitor interacts directly or indirectly with the biosynthesis of ACC and ethylene has not been mentioned yet. Therefore, one of the potential inhibitors of ethylene biosynthesis is the ability to limit ACC synthase and ACO activities upon the biosynthetic intermediate ACC is the essential solution (Schaller *et al.*, 2017; Xuewen *et al.*, 2011). Because inhibition of ethylene production in various climacteric fruits was not enough to significantly change ripening and post-harvest behavior (Pech *et al.*, 2008), the application of AVG inhibitors to Booth7 Avocado has not been studied. Hence, in this study, Booth7 Avocado at the pre-climacteric interval was chosen to evaluate the impacts of three AVG concentrations on ethylene production and fruit quality during the storage period at 8°C temperature. Finally, the inhibitory results of AVG responding to three different treatments of AVG concentration range from 200, 400, and 600μL L^{-1} determined differential changes in the physiological and biochemical properties of avocado booth7. This likely led to a proposed method by AVG treatments for delaying fruit ripening. The remaining sensory properties of Booth7 Avocado under commercial temperatures are essential for growers in Vietnam and other countries.

MATERIALS AND METHODS

Materials and Preservation Treatment of Booth7 Avocado

Booth7 Avocado materials were hand-harvested at their physiological maturity of 230 to 240 days after flowering in the Daklak province of Vietnam and immediately transported to the laboratory (average weight of 350 g and length of 25cm for Booth 7 Avocado). The selected avocados were uniform in size, color, and absence of defects. They were divided randomly into four treatments with an equal total number for each treatment and then placed in shipping boxes (eight Booth7 avocados per box). The AVG powder was diluted to make four different concentrations for four incident applications 1) AVG 0 μL L^{-1}, 2) AVG 200 μL L^{-1}, 3) AVG 400 μL L^{-1}, and 4) AVG 600 μL L^{-1} were used for examination with three replicates. Four treatments were sealed in 1,000 L airtight plastic jars (0.15 mm thickness) tents and immediately stored at 8°C and 85% - 90% humidity. Each treatment was collected in triplicate of 3-day cycle repeats to determine ethylene production, fruit firmness, ACC content, ethylene biosynthesis enzymes, mass loss, and spoilage ratio. Each treatment was halted when its spoilage rate exceeded 20%, compared to that in Booth7 Avocado at the control treatment.

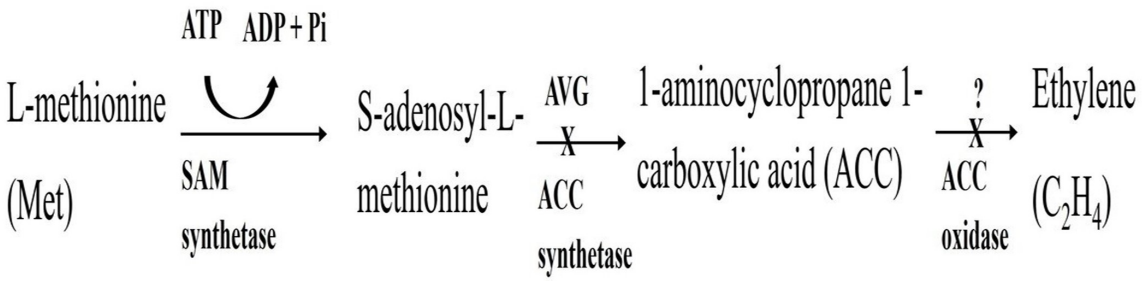

Figure 1: The proposed activity mechanism of ACS and ACO enzymes from precursor L-methionine (Met) to ethylene production and the site of action for aminoethoxyvinylglycine (AVG) inhibitor at the activity of ACS enzyme

Method for Determining Respiration Rates and Ethylene and ACC Concentrations

Ethylene production, ACC concentrations, and respiration rates were determined for Booth7 Avocado fruits using a static system. At each treatment, three fruits were weighed and put in 0.5 L jars. Carbon dioxide concentrations (ml CO_2 kg^{-1} h^{-1}) and ethylene production (μl C_2H_4 kg^{-1} h^{-1}) in the jar headspace were determined using two gas analyzers (Barker, 2002). At the assessed phase, samples were maintained at 7°C with an interval of 7 hours to determine the parameters. Because ACC is a precursor material of ethylene, its concentration was measured based on the reported method (Concepcion *et al.*, 1979). This method's conversion efficiency to ethylene was approximately 80%; therefore, the ACC content in each sample was measured using an ICA 56 Dual Analyzer (Japan). Finally, the ACO activity was refined, consistent with the modified method of Moya-León and John, with a 10 mM ACC substrate (Moya-Leon *et al.*, 1994).

Physicochemical Analysis of Booth7 Avocado

The titratable acidity: The total acid number (TAN) in fresh fruits was determined by titration of a known quantity of sample (10 mL) against 0.1 N sodium hydroxide using 1% phenolphthalein solution as an indicator to a persisting fade pink point. TAN values were expressed as percent anhydrous citric acid using the underlying formula 1 (AOAC, 1994).

Estimating total sugars: Reduced sugar was estimated using Bertrand's method (AOAC, 2000). The contents of monosaccharides in the raw material were then evaluated indirectly upon account of the reduced sugars in the redox reactions with the metal ions. An aliquot (100 mL) of the filtrate containing reducing sugars was hydrolyzed using 10 mL of 50% hydrochloric acid at room temperature for 24 h. After the hydrolase period, this solution was neutralized using 40% sodium hydroxide and phenolphthalein as an indicator; the reaction volume was made up to 250 mL and then titrated against Fehling's solution. The total sugars were calculated using the formula (Sabeera *et al.*, 2016).

Percentage Evaluation of Mass Loss and Spoilage

The sample masses in each treated experiment were weighted using an analytical balance during the 30-day survey. The measured results were used to calculate the ratio of mass loss when compared to the original mass. The mass loss percentage (M) was counted using the following formula:

$$M(\%) = \frac{\text{prior mass} - \text{posterior mass}}{\text{prior mass}} \times 100\% \quad (2)$$

The spoilage percentage was determined by measuring the area of the damaged surface of the fruit. Four levels were indexed in the descriptions, including:

- Level 0: The percentage of fruit surface deterioration is 0%.
- Level 1: The percentage of fruit surface deterioration is 25%.
- Level 2: The percentage of fruit surface deterioration ranging from 25% to 33.3%.
- Level 3: The percentage of fruit surface deterioration ranging from 33% to 50%.

Statistical Analysis

Experiments were conducted using a random design. All statistical analyses were performed with the

$$TAN(\%) = \frac{\text{Tire value} \times \text{Normality of alkali} \times \text{Volume made} \times \text{Weight of sample}}{\text{Volume of sample} \times \text{Weight or Volume of sample} \times 1000} \times 100 \quad (1)$$

The spoilage percentage (T) was calculated using the following formula:

$$T(\%) = \frac{1 \times \text{Level 1 of number} + 2 \times \text{Level 2 of number} + 3 \times \text{Level 3 of number}}{\text{Total number of three levels}} \times 100\% \quad (3)$$

Statistical Package for the Social Sciences (SPSS) 23 software. The data were subjected to a one-way analysis of variance (Nenko Nenov 1). Mean separations were performed using Ducan's test. Differences at $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of AVG Treatments on Respiration Rate, Production Abilities of ACC Precursors, Ethylene Production, and Qualification of The ACO Enzyme Activity of Booth7 Avocado in Days After Storage (DAS)

Because typical climacteric characteristics with high ethylene production occur at a fast-ripening rate and short shelf life, the proper regulation of respiration rate and control of the production of ACC substances and ethylene hormone sequentially help to prolong the shelf life and increase economic efficiency. Hence, respiration rates in four treatments showed that the CO_2 intensities of all treatments decreased together in the first 6 days of the storage period but then increased slightly in the next days (Fig. 02a and Table 02). These results showed no differential changes in the overall trend of respiration rates in all treatments from the beginning date until the 15th DAS. Subsequently, the Booth7 Avocado fruits in the control treatment showed a rapid increase in respiration rate and approached the highest CO_2 intensity of 24.20 μmol CO_2 kg^{-1} h^{-1} on the 18th DAS. This result contrasted with Booth7 Avocado managed in three AVG treatments (200, 400, and 600 μL L^{-1}) by their respiratory rates, which remained slow level in the beginning 15 days of DAS. Treating the lowest concentration of AVG (200 μL L^{-1}), changes in respiratory rate were impressed by its maximum value on the 21st DAS of 22.59 μmol CO_2 kg^{-1} h^{-1}. In contrast to higher AVG treatments of 400 μL L^{-1} and 600 μL L^{-1}, the CO_2 intensities slowly increased until the 21st DAS. Differential variations were observed on the 24th DAS when the CO2 intensities were at the highest levels, with relevant values of 20.67 μmol CO_2 kg^{-1} h^{-1} and 20.00 μmol CO_2 kg^{-1} h^{-1} ($p\leq0.05$) (Table 01).

Since both ethylene and CO_2 concentrations were also products in a redox reaction from ACC precursor by enzyme ACC oxidase (ACO), their contents have been refined as products of the catalytic activity of ACO (Schaller *et al.*, 2017; Sitbon *et al.*, 2011). The same ACC concentration of 10 mM existed in all treatments in the initial period; however, the accounts of the converted ethylene concentrations upon the activity of the intracellular ACO enzyme in all treatments were different following the storage period. Figure 02c shows that ACO enzyme activity in all treatments had not changed significantly at the 9th DAS. Unlike the results of ACC production, the ACO enzyme activity in the control treatment revealed that the operations of both ACO and ACS enzymes were not concomitant when the highest amount of the highest produced ACC amount of the ACS enzyme occurred on the 15th DAS. Upon converted ethylene, the activity of the ACO enzyme attained the highest produced ACC amount of the ACS enzyme on the 18th DAS. For the control treatment, the heightened level was 23.78 nmol C_2H_4. g^{-1} on the 18th DAS and followed by a rapid decrease if the sequential d to follow-up completed experiments before the 27th. In contrast, the ACO enzyme activities in the AVG treatments were not appraised as high, the activity was kept mostly at levels lower than the control treatment. The ACO enzyme activity in the 200 μL L^{-1} AVG treatment slowly increased during the initial 15 days of DAS, after which there was a significant increase approaching the best ACO activity on the 21st DAS by the highest level of 21.68 nmol C_2H_4 g^{-1}. Before the 21st DAS, the number of converted ethylene products in the higher AVG treatments remained at lower levels. The best ACO enzyme activity was recorded on the 24th DAS at the highest levels of 20.02 nmol C_2H_4 g^{-1} or 19.69 nmol C_2H_4 g^{-1} (Fig. 02c). In particular, the amounts of created ethylene in high AVG treatments showed no significant differences ($p\leq0.05$) (Table 01).

The ACC precursor is a substrate that plays an important role in ethylene biosynthesis. After each ACC precursor is produced by the activity of the ACS enzyme, it is converted to ethylene by the catalytic activity of another intracellular ACO enzyme in the cell (Yang, 1984).

Table 1: Differential effects of AVG treatments on four typical indicators including respiration rate, ACC concentration, ethylene concentration and ACO activity on three climacteric fruits such as Booth7 Avocado, Apple (Burhan *et al.*, 2015) and Peach (Hayama *et al.*, 2008) during DAS

Storage time (days)	AVG treatment (μL. L^{-1}) on Booth7 Avocado in this study				AVG (μL. L^{-1}) on Apple			AVG (μL. L^{-1}) on Peach	
	0	200	400	600	0	200	400	0	150
Respiration rate (μmol.kg^{-1}.h^{-1})									
0	16.0	16	16	15.8					
9	11.00^{a}	11.49^{a}	10.63^{a}	10.23^{a}					
15	15.67c	14.0^{b}	12.7^{a}	12.0^{a}					
18	24.20^{c}	17.30^{b}	15.00^{a}	13.37^{a}					
21	16.78^{a}	22.59^{b}	17.67^{a}	17.52^{a}					
24	12.96^{a}	16.61^{b}	20.67^{c}	20.00^{c}					
27	-	13.64^{a}	16.66^{b}	17.03^{b}					
30	-	-	13.98	14.80					
ACC (nmol C_2H_4.g^{-1})									
0	0.33	0.28	0.31	0.26					
9	2.24^{c}	1.74^{b}	1.07^{a}	0.91^{a}					
15	5.29^{c}	3.54^{b}	2.36^{a}	1.92^{a}					
18	4.27^{c}	5.08^{b}	3.63^{a}	3.49^{a}					
21	3.66^{a}	3.94^{a}	4.78^{b}	4.64^{b}					
24	3.04^{a}	3.42^{b}	4.05^{c}	3.95^{c}					
27	-	2.93^{a}	3.32^{a}	3.24^{a}					
30			2.81	2.87					
Ethylen (nmol C_2H_4.kg^{-1}. h^{-1})									
0	0.44	0.43	0.39	0.33	9.71.10^{5a}	7.27.10^{5d}	7.80.105^{cd}	-	-
9	0.97^{b}	1.09^{b}	1.02^{a}	0.91^{a}				4.13	8.26
15	13.55^{c}	10.77^{b}	9.11^{b}	6.54^{a}	1.80.10^{6a}	1.41.10^{6b}	1.22.10^{6c}	-	-
18	19.66^{c}	12.69^{b}	10.53^{a}	9.14^{a}					
21	11.74^{a}	17.87^{b}	13.88^{a}	13.38^{ab}	2.92.10^{6a}	1.98.10^{6c}	1.33.10^{6e}		
24	9.45^{a}	12.79^{b}	16.83^{c}	15.74^{c}					
27	-	9.60^{a}	11.88^{b}	12.65^{b}					
30	-	-	10.29	10.13					
ACO activity (nmol C_2H_4.g^{-1})									
0	3.83	3.85	3.76	3.64					
9	11.02^{a}	9.77^{ab}	8.93^{ab}	8.21^{ab}					
15	16.69^{d}	13.69^{c}	11.85^{b}	10.93^{a}					
18	23.78^{c}	16.83^{b}	12.99^{a}	12.30^{a}					
21	17.81^{a}	21.68^{b}	16.17^{a}	15.08^{a}					
24	12.56^{a}	16.32^{b}	20.02^{c}	19.69^{c}					
27	-	13.01^{a}	17.88^{b}	16.81^{b}					
30		-	13.53	14.05					

The different letter shows statistical differences by Ducan's test at p 0.05

The effects of four AVG treatment concentrations on variations in ACC contents during storage periods are shown in Figure 02b. All treatments also revealed changes significantly in three days, with little recorded fluctuation from 0.26 nmol C_2H_4 g^{-1} to 0.33 nmol C_2H_4 g^{-1}. It is easy to recognize that ACC concentrations in the control treatment increased rapidly to the highest value of 5.29 nmol C_2H_4 g^{-1} on the 15th DAS, but then decreased by 20% of the previous concentration on the 18th DAS and continued to decrease rapidly in the following days. However, in Booth7 Avocado at the AVG 200 μl L^{-1} treatments showed a remarkable event in which ACC production reversed on 3 days, with its highest level of 5.08 nmol C_2H_4 g^{-1} on the 18th DAS. In contrast to both high AVG treatments (AVG 400 μL L^{-1} and AVG 600 μL L^{-1}), the ACC contents always remained with the lowest levels from the initial date to the 18th DAS ($p \leq 0.05$) and then the highest ACC levels were produced on the 21st DAS with values of 4.78 nmol C_2H_4 g^{-1} and 4.64 nmol C_2H_4 g^{-1}. Especially in the period from the 27th DAS, the ACC levels produced in the three AVG treatments were not significantly different ($p > 0.05$) (Table 01).

Finally, the measured ethylene contents in all the treatments were very low from the first day to the 9th DAS; there were no significant differences among the treatments. However, a basic feature of the climacteric period of Booth7 Avocado was seen on the 18th DAS in the control treatment when the ethylene content reached the highest level of 19.66 nmol C_2H_4 kg^{-1} h1. Its value immediately declined on the next day. In contrast to the control treatment, fruits applied at the AVG treatments showed remarkably lower ethylene production during the natural climacteric period of Booth7 Avocado from the initial date to the 18th DAS. As shown in Figure 02d, the impacts of two higher AVG concentrations also demonstrated its useful inhibition by dragging the climacteric peak of Booth7 Avocado to six days, while the climacteric peak in the AVG of 200 μL L^{-1} treatment delayed the process only by three days compared to the control treatment (p 0.05). Moreover, the ethylene concentrations at the peak of AVG 200 μL L^{-1} treatment was 2 nmol C_2H_4 kg^{-1} h^{-1}, while in the higher AVG treatments were 3 nmol C_2H_4 kg^{-1} h^{-1} and 4 nmol C_2H_4 kg^{-1} h^{-1}, and those contents were less than those in the control treatment (Table 01). These results could be considered an effective inhibitory threshold for limiting ethylene production in Booth7 Avocado with AVG concentrations from 200 μL $L^{-1}$to 600 μL L^{-1}(Fig. 02d).

The Booth7 Avocado is a climacteric fruit characterized by a burst in ethylene production and respiration during ripening; fruit ripening often occurs on the 5th to 7th days after harvest. Previous studies have evaluated the inhibition of AVG to the intensity of ethylene in some climacteric fruits but have not indexed in Booth7 Avocado until now. Moreover, the overall results showed that the AVG inhibitor treatment method offered a potential solution for delaying ripening during the storage period by reducing the biosynthesis of ACC and ethylene. The efficacy of AVG was proven by statistical analysis, which showed a highly significant improvement in the quality of Booth7 Avocado compared to that in the control treatment. Following to the observed data in Figure 02 and Table 01 showed that the AVG inhibitor affected the ACS intracellular synthase activity, and interfered with the respiration rate, the ACO nitrocellulose enzyme activity, and ethylene production. The observable differences in the ACO enzyme activities in all treatments from the 9th date until the 24th DAS showed both CO_2 and ethylene, with similar trends during the same period. In contrast to the more substantial changes in the control treatment, few fluctuations were observed in the concentrations of CO_2 and ethylene in the AVG treatments.

A comparative analysis of differential effects of AVG treatments on four typical indicators, including respiration rate, ACC concentration, ethylene concentration, and ACO activity on three climacteric fruits (avocado, apple, and peach), are summarized in Table 01. Table Three treated fruits showed inhibitory ability on ethylene content. Because each fruit responded to a different degree using attendance inhibitors, on the 9th DAS, the ethylene in three AVG treatments had increased by about 0.6 – 0.7 nmol C_2H_4 kg^{-1} h^{-1}, while those of the treated peach were 10 times higher at 8.26 nmol C_2H_4 kg^{-1} h^{-1}. Inhibition of AVG to the ethylene contents was more obvious on the 21st DAS at the same AVG concentration of 400 μL L^{-1}. The ethylene content of Booth7 Avocado was 16.83 nmol C_2H_4 kg^{-1} h^{-1}, the apple was 1.33.106 nmol C_2H_4 kg^{-1} h^{-1}. Besides, the recorded contents of CO_2 and ethylene in both the 400 μL L^{-1} AVG and the 600 μL L^{-1} treatments had almost no impact of high AVG concentrations on the production of CO_2 and ethylene ($p > 0.05$). These observed results demonstrated that the activities of intracellular ACO enzymes of the AVG treatments remained at low levels. Finally, treatments using higher AVG concentrations revealed stronger inhibitions of ethylene biosynthesis for the three treated fruits. Besides, variations in the contents of these components participated in events relating to climacteric characteristics of the Booth7 Avocado.

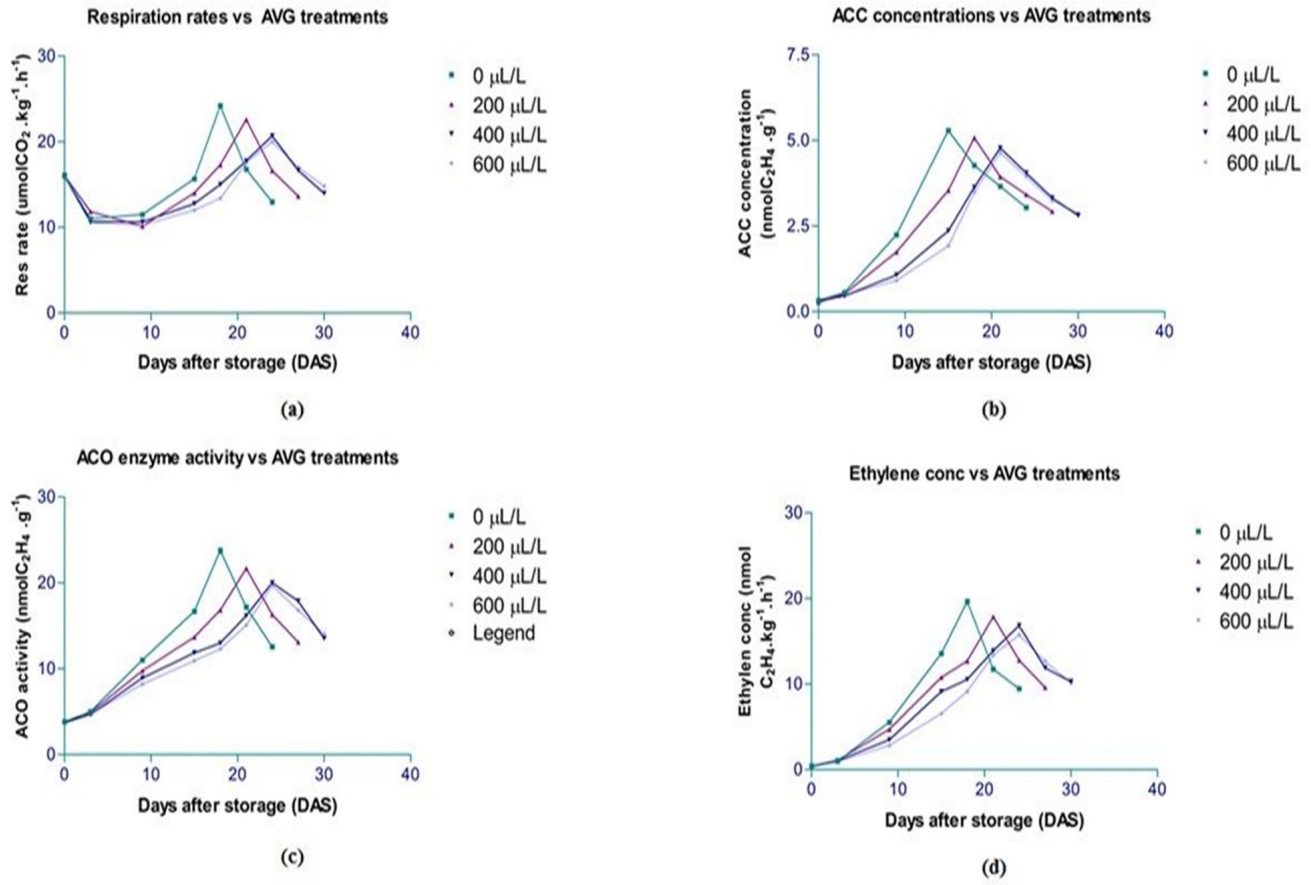

Figure 2: Effect of AGV treatments on respiration rate and the production abilities of ACC precursors and ethylene production and qualification of the ACO enzyme activity of Booth7 Avocado during DAS. (a) Effect of AGV treatments on respiration rate; (b) Effect of AGV treatments on accumulations of ACC precursors; (c) Effect of AGV treatments on the activity of ACO enzyme; (d) Effect of AGV treatments on ethylene productions. (a) Effect of AGV treatments on respiration rate; (b) Effect of AGV treatments on accumulations of ACC precursors; (c) Effect of AGV treatments on the activity of ACO enzyme; (d) Effect of AGV treatments on ethylene productions

Development in both climacteric and non-climacteric fruits showed parallel changes in various metabolic processes involving sugar accumulation by the degradation of starch and changes in total acid and ethylene production. Therefore, the influences of AVG treatments on the total sugar content of Booth7 Avocado were recorded and assessed during this study's storage period. Table 02 shows the significant differences in the chemical compositions of Booth7 Avocado for the three AVG treatments.

The total sugar content of all treatments fluctuated a little in the beginning 9 days of DAS. Then, a change in all samples occurred on the 15th DAS as the total sugar contents of all treatments gradually decreased until the 30th DAS. The total sugar content of the control treatment and the AVG 200 μl L^{-1} treatment decreased each day from the 15th to the 21st DAS. However, overall assessments showed no differences in variations in the total sugar content ($p>0.05$). The highest total sugar contents in the higher AVG treatments revealed the impacts of high AVG concentrations on restricted losses of the total sugar contents ($p\leq0.05$). The control treatment ended the experiments on the 27th DAS because its spoilage rate exceeded 20%, while three AVG treatments were performed continuously in the next days of DAS. The remaining total sugar in the three AVG treatments (AVG 200 μL L^{-1}; AVG 400 μL L^{-1} and AVG 600 μL L^{-1}) were 1.17%, 1.28%, and 1.48%, respectively. At this time, the total sugar content of the three AVG treatments on the 27th DAS was not significantly different ($p>0.05$) (Table 02).

Organic acids are vital materials in respiration, metabolism, and energy production, as the initial stage showed that the total acid amounts of all treatments in the initial stage had an average content of 0.27%. Still, on the next day, these contents showed an overall decline in all treatments (Fig. 03). Inhibition of AVG inhibitor on the consistency of organic acid was reflected in differential variations of the total acid content in three AVG treatments from the 15th d to the

Table 2: Effect of AVG treatments at the pre-harvest interval on the contents of total sugar and total acid on Booth7 Avocado (this study), Booth7 Avocado (Blakey *et al.*, 2015) and Apricot (Defilippi *et al.*, 2009) during DAS

Storage time (Udayaprakash)	AVG treatment (μL L^{-1}) to Booth7 Avocado in this study				Booth7 Avocado	Apricot	
	0	200	400	600		Yellow-green	Yellow
Total sugar (%)							
0	3.14	3.08	3.12	3.15	-		
15	1.92^{a}	2.10^{a}	2.46^{b}	2.51^{b}	1.4		
21	1.45^{a}	$1,53^{a}$	$1,90^{b}$	$2,15^{b}$			
24	1.26^{a}	$1,29^{ab}$	$1,59^{bc}$	$1,75^{c}$			
27	-	$1,17^{a}$	$1,28^{a}$	1,48			
30	-	-	1.14	1.32			
Total acids (%)							
0	0.27	0.27	0.28	0.28		0.19^{aA}	0.19^{aA}
15	0.085^{a}	0.099^{a}	0.131^{b}	0.138^{b}		0.16^{aA}	0.16^{aA}
21	0.051^{a}	0.071^{ab}	0.098^{bc}	0.113^{c}			
24	0.046^{a}	0.052^{a}	0.075^{b}	0.092^{b}			
27	-	0.042^{a}	0.066^{ab}	0.080^{b}		0.16^{aA}	0.16^{aA}
30	-	-	0.051	0.058			

Table 3: Effect of AVG treatments at pre-harvest interval to losses of mass and spoilage of Booth7 Avocado (this study) and Effect of two1-MCP treatment (0 and 75 ppb) on Booth8 Avocado (Adrian *et al.*, 2015) during DAS

	Mass loss ratio (%)				Spoilage ratio (%)		
	21d	24d	27d	30d	24d	27d	30d
Treatments							
AVG 0μL. L^{-1}	$4,66^{d}$	$5,09^{b}$	-	-	20	-	-
AVG 200μL. L^{-1}	$3,83^{c}$	$4,52^{b}$	4,82	-	11.11	17.78	-
AVG 400μL. L^{-1}	$3,35^{b}$	$3,71^{a}$	4,32	4,67	6.67	13.33	16.67
AVG 600μL. L^{-1}	$2,69^{b}$	$3,53^{a}$	4,12	4,38	7.11	15.56	19.44
1-MCP 0 ppb (Adrian D. Berry, 2015)			5.92				
1-MCP 75ppb (Adrian D. Berry, 2015)			6.09				

sequential days ($p \leq 0.05$). For the control treatment, the total acid content recorded on the 24th DAS was 0.046%, and then its experiment was stopped sooner because its spoilage rate exceeded 20%. However, for the three AVG treatments, the spoilage rates were not less than 10%. Hence, the relevant total acids at three AVG treatments collected on the 27th DAS values were 0.042%, 0.066%, and 0.080%. The influence of AVG concentrations on the chemical composition of Avocado booth7 was demonstrated on the 30th DAS when the spoilage rate in the lowest AVG treatments (200 μL L^{-1}) surpassed 20%, with two higher AVG concentrations under 20%.

The impacts of AVG treatment on Booth7 Avocado at the pre-harvest interval reflected a reducible restriction on the contents of total acid and total sugar. The total sugar content in the first interval corresponded to relevant ranges of 3.08% to 3.14%. These concentrations were approximately two times higher than the Booth7 Avocado in the previously reported studies. These results showed that the flesh firmness of Booth7 Avocado at pre-treatment could maintain total sugar content during DAS. Furthermore, a study of the post-harvest quality of apricots refined variations in total acid content between collected samples at both pre-harvest and harvest intervals, with no differences during 27 days of the period storage at 0°C (Defilippi *et al.*, 2009). Our experimental results demonstrated that AVG treatment at the pre-harvest interval significantly limited the changes in the total acid and sugar content, especially with high AVG treatments of 400 μL L^{-1} and 600 μL L^{-1}. These results surpassed other treatments. Alternatively, the AVG treatment method could extend shelf life and maintain the high total acid content of Booth7 Avocado.

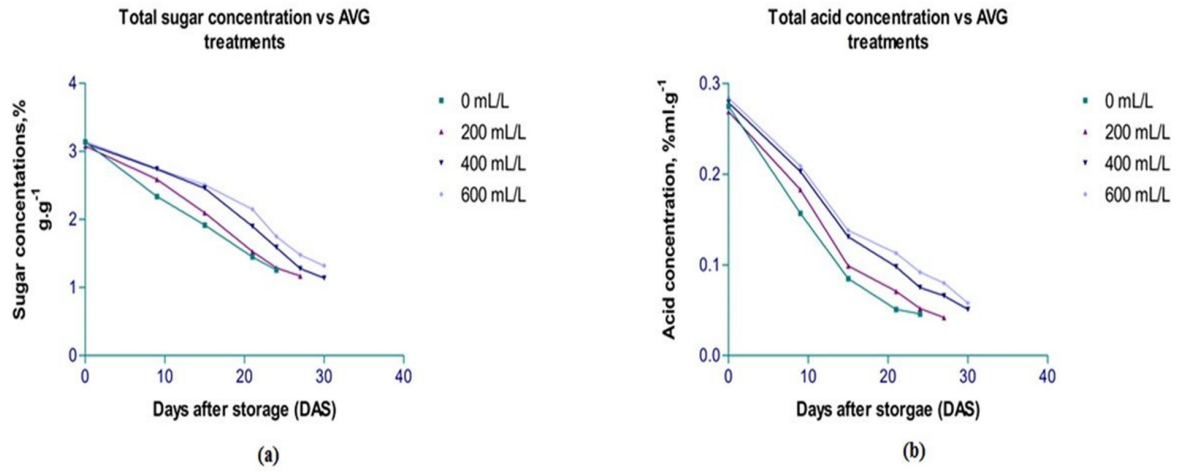

Figure 3: Effect of AVG treatments at the pre-harvest interval on the contents of total sugar and total acid on Booth7 Avocado during DAS. (a) Effect of AGV treatments on the total sugar content; (b) Effect of AGV treatments on the total acid content

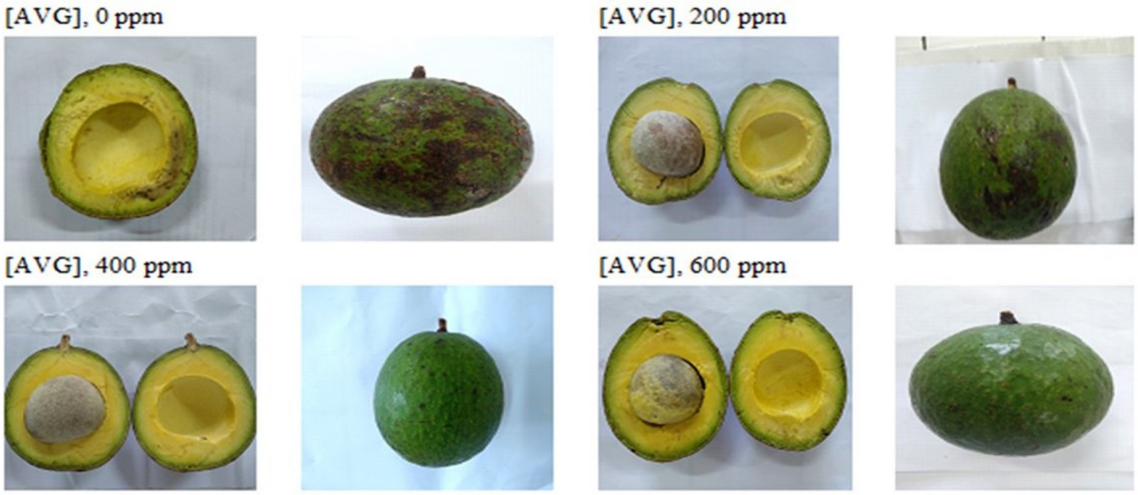

Figure 4: Effect of AVG treatments at pre-harvest interval on the spoilage of Booth7 Avocado at 24th DAS. (a): The spoilage of Booth7 Avocado in the control treatment; (b): The spoilage of Booth7 Avocado in the AVG concentration of 200 μL L^{-1}; (c) The spoilage of Booth7 Avocado in the AVG concentration of 400 μL L^{-1}; (d): The spoilage of Booth7 Avocado in the AVG concentration of 600 μL L^{-1}

Effect of AVG Treatments at Pre-harvest Intervals on Losses of Mass and Spoilage Rate of Booth7 Avocado during The Storage Period

The life cycle of higher plants comprises several stages, from fruit development to ripening and the commencement of senescence. Evidence about the effect of AVG treatments at the pre-harvest interval on losses of mass and spoilage of Booth7 Avocado after harvest has not been examined. Under storage conditions of 8°C and humidity of 85-90%, a gradual mass loss was observed in all treatments on the first 9 days of DAS; however, the mass losses increased quickly on the sequential 3 DAS (data not shown). The increase in mass losses was associated with a gradual reduction in the amounts of total sugar and acid in Booth7 Avocado pulp. On the 21st DAS, the mass loss rate showed significant differences in all treatments ($p \leq 0.05$). Although the respiration rate of Booth7 Avocado of AVG 200 μL L^{-1} treatment had reached the maximum value, its mass loss ratio was 3.83%, lower than the control treatment of 4.66%. Ripening progressed until Booth7 Avocado reached ripeness; hence, mass losses in all treatments were recorded continuously in the following days. Observed events occurred on the 27th DAS when the mass loss ratios revealed no differences in the three AVG treatments ($p > 0.05$). Still, no data was collected for the control treatment because its experiments had been stopped entirely beforehand.

The spoilage rates were manifested by dark bruises

in the skin and flesh, a loose flesh structure, and the flesh separated from the shell. Based on the collected images and results of the ripening processes of Booth7 Avocado, spoilage in the control treatment was sequentially faster than in the AVG treatments. Booth7 Avocado was treated with high AVG at 400 μL L^{-1} and 600 μL L^{-1}, the spoilage rates were the lowest. However, the sensory properties of Booth7 Avocado are at the highest AVG concentrations (600 μL L^{-1}) and showed fibrous texture formation and lower putridity at the end of storage compared to that of the AVG at 400 μL L^{-1}(Fig. 04).

In summary, higher AVG concentrations inhibited physiological and biochemical changes during storage. Based on ANOVA statistics, there were no significant differences in inhibition ability at the 5% level ($p>0.05$) on the 30th DAS from the three AVG treatments in Table 03.

Due to the ripeness peak of Booth7 Avocado in the control treatment was observed on the 18th DAS resulting in why its spoilage rate increased significantly on the 24th DAS by over 20%. In contrast to the AVG treatments, the extent of mass losses was from 3.53% to 4.52%, and the spoilage ratio ranged from 7.11% to 11.11% on the 24th DAS (Table 03). On the 27th DAS, there were no statistical differences among Booth7 avocados in the three AVG treatments ($p>0.05$). Consequently, mass losses of treated AVG Booth7 Avocado in Table 03 were 2% less than both the 1-MCP-treated Booth8 Avocado and the Monroe avocado on the same day. Besides, maintaining firm flesh in Booth7 Avocado before AVG treatment in our study resulted when the sugar content fluctuated very little in the first 9 days and thus the application of AVG had caused more substantial impacts on the total acid concentration in Booth7 Avocado. Comparative studies with a known inhibitor, such as 1-MCP, on two avocado hybrids (Monroe and Booth8), reported respiratory peaks on the untreated sample on the 15th DAS and the treated 1-MCP sample on the 21st DAS (Adrian *et al.*, 2015). Still, ethylene levels peaked in both samples simultaneously on the 16th DAS. The data in our study showed that an optimal significant inhibition of AVG at 400 μL L^{-1} resulted from the lowest mass loss. The spoilage ratio was 16.67% on the 30th DAS, while the control treatment exceeded 20% on the 24th DAS. Furthermore, a new study concluded that the AVG inhibitor does not have a negative impact on the number of bioactive compounds in plum fruits (Burhan *et al.*, 2012).

CONCLUSION

In this study, the activity of intracellular ACS showed no increase concomitant with the catalytic activity of intracellular ACO enzymes to convert the ACC precursor to ethylene production was observed firstly in Booth7 Avocado. Besides, in comparison of the structural characteristics of AVG in interaction with two S-adenosyl-L-methionine and ACC substrates of ACS and ACO enzyme of Booth7 Avocado, we recognized that the AVG inhibitor and both S-adenosyl-L-methionine and ACC substrates were polar compounds with a carboxyl group at the end. It could be explained by the carboxyl group is the functional group that presents vital properties for forming hydrogen bonds with both ACS and ACO enzymes, and the properties of the carboxyl group are highly electronegative and weakly acidic. In contrast to the 1-MCP inhibitor, cyclopropane does not involve a functional group. Taken together, based on the structural insight of AVG and S-adenosyl-L-methionine and ACC, we propose new findings about AVG's role in the inhibition of the activities of both ACS and ACO enzymes, including changes in reducing the contents of chemical compounds during the storage period compared to that in the control treatment.

Because fruit softening is an integral part of the ripening process and is associated with textural changes by the alternation of the chemical compositions of fruits, this study revealed that pre-harvest AVG treatments effectively delayed the ripeness and softness of Booth7 Avocado in Vietnam. Significantly, in this study, a valid concentration of 400 μL L^{-1} provided the optimal AVG concentration to inhibit ACO enzyme activity and ethylene production, and its corollary is retardation of the ripening period to 6 days and restricted the spoilage rate of Booth7 Avocado by 16.67% at 30 days and 8°C temperature. This is consistent with strategies to find one directed suppression of ethylene production using stronger inhibitions resulting from knocking down the activity of ACS and ACO enzymes (Pech *et al.*, 2008). Therefore, a combination solution using AVG treatment was a potential inhibitor to effectively suppress ethylene production and delay fruit softening by limiting changes during ripening in Booth7 Avocado.

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