Effects of Abscisic Acid on Bioactive Compounds and Postharvest Quality of Climacteric and Non-Climacteric Fruit

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ABSTRACT

Abscisic acid (ABA) is a growth regulatory substance that plays an important role in various processes in the cell, such as plant adaptation to environmental threats, seed development, dormancy, budding, plant development, and fruit ripening. Another important role of abscisic acid is increased antioxidant capacity, total phenol content, total carotenoid, and anthocyanin content. The content of bioactive compounds such as antioxidants is a parameter of fruit quality that can decrease due to improper postharvest handling. Many postharvest treatments have been conducted to maintain or delay the declining of bioactive compounds in fruits. This current research was done to determine the various abscisic acid concentrations on papayas and cucumbers' bioactive content. The experiment was arranged in a completely randomized design with three replications. The data were expressed as mean ± standard deviation and analyzed using SAS (Statistical Analysis Software). Significant differences between means were determined by the Student's t-test, with p-values less than 0.05 considered significant. Results of the research regarding color testing, DPPH, total phenol content, and total soluble solids showed that abscisic acid with 380 μMol or 100 mg/l concentration could increase the total phenol content, antioxidant capacity, and total soluble solids in papayas (climacteric fruit) during the ripening. However, applying abscisic acid with 100 μMol on cucumbers (non-climacteric fruit) showed no significant effect on total phenol, antioxidant capacity, and soluble solids compared to the control group. The results showed that during the ripening of climacteric fruit, ABA enhanced the content of bioactive compounds and improved postharvest quality. This effect was not observed in non-climacteric fruit.

Keywords: Antioxidant, Cucumber; Papaya; Phenolic compound, Soluble solids

ABSTRAK

Asam absisat adalah senyawa pengatur pertumbuhan yang memiliki peranan penting dalam berbagai proses dalam sel, seperti kemampuan adaptasi tanaman terhadap cekaman lingkungan, perkembangan biji, dormansi, pertunasan, perkembangan tanaman dan pematangan buah. Peran penting lain dari asam absisat adalah pada peningkatan kapasitas antioksidan, kadar total fenolik, kadar total karoten, dan kadar antosianin. Kandungan komponen bioaktif seperti antioksidan merupakan parameter dari kualitas buah, yang dapat berkurang akibat penanganan pascapanen yang kurang tepat. Berbagai upaya penanganan pascapanen telah banyak dilakukan untuk mempertahankan atau menghambat penurunan kandungan senyawa bioaktif pada buah. Penelitian ini dilakukan utuk menentukan pengaruh konsentrasi asam absisat terhadap senyawa bioaktif pada pepaya dan timun. Penelitian disusun dalam Rancangan Acak Lengkap dengan tiga ulangan. Data dianalisis menggunakan SAS (Statistical Analysis Software) dan diekspresikan dalam rata-rata ± Standar deviasi. Beda nyata antar rata-rata ditentukan menggunakan Student's t-test, dg nilai p kurang dari 0,05. Hasil pengujian terhadap warna, aktivitas antioksidan, kadar total fenolik, dan total padatan terlarut menunjukkkan bahwa pada konsentrasi 380 μMol atau 100 mg/l, asam absisat mampu meningkatkan kadar total fenolik, kapasitas antioksidan dan total padatan terlarut pada pepaya saat tahap pematangan. Akan tetapi, aplikasi dengan konsentrasi 100 μMol pada timun tidak menunjukan perbedaan signifikan pada total fenolik, kapastias antioksidan dan total padatan terlarut dibandingkan sampel kontrol. Hasil penelitian menunjukkan bahwa selama pematangan buah klimakterik, ABA mampu meningkatkan kandungan senyawa biokatif dan meningkatkan kualitas pasca panennya, tetapi tidak pada buah non klimakterik.

Kata kunci: *Aktioksidan; Padatan terlarut; Pepaya; Senyawa fenolik; Timun*

INTRODUCTION

Due to their health benefits and nutritional content, fruits and vegetables are considered functional foods. Polyphenols are the most widely-known antioxidants, and they are primarily found in fruits and vegetables, which makes consuming fruits and vegetables regularly very beneficial for human health (Asghar et al., 2016). Most fruits and vegetables contain antioxidant, which is a

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health-beneficial compounds, aside from their external qualities such as color, taste, shape, and size (Villa-Rodriguez et al., 2015; Yunusa et al., 2018)

Papaya is a climacteric fruit with a high level of iron and calcium. Various vitamins, such as vitamins A, B, and C (ascorbic acid), are found in papaya. These compounds are typically useful for boosting the immune system. Papaya also contains carpaine alkaloids, which can decelerate heart rate and help lower blood pressure (Arshad et al., 2020; Asghar et al., 2016; Baidya & Sethy, 2020). Cucumber, one of the examples of non-climacteric fruits, is a commodity that is recommended to be widely consumed, especially in the fresh form, to boost the immune system. Cucumber contains erepsin enzymes, Vitamins B1 and C, proteolytic enzymes, and rutin flavonoids that act as antioxidants. Several studies report the high antioxidant capacity of cucumber as it contains phenolic compounds, proanthocyanidins, and flavonols (Kumar et al., 2010; Sotiroudis et al., 2010; Yunusa et al., 2018).

Abscisic acid (ABA) is a growth regulator that is involved in a variety of processes in plant cells, such as adaptation to environmental stresses, seed development, dormancy, germination of plant growth and development, and fruit ripening (Vishwakarma et al., 2017; Yang & Feng, 2015). During fruit ripening, ABA can increase ethylene production, affect respiration, fasten pigment and color changes, play a role in the metabolism of phenol compounds, enhance antioxidant capacity, and increase total phenol, carotenoids, and anthocyanins levels (El-Mogy et al., 2019; Franzoni et al., 2016; Setha, 2012). With the growing need for bioactive compounds and antioxidants to improve the immune system, further investigation into the contribution of abscisic acid in maintaining bioactive compounds to determine its potential for human health is needed.

This research aimed to assess the impact of exogenous ABA application on the bioactive compound content and postharvest quality of both climacteric and non-climacteric fruits. This study evaluated the antioxidant activity, total phenolic content, and other relevant quality parameters of papaya (climacteric) and cucumber (non-climacteric).

MATERIAL AND METHODS

Application of Abscisic Acid

Fruits were washed and dried, then sprayed using a mixture of abscisic acid (380 µM for papaya and 100 µM for cucumber). The concentration used was of the optimum level according to the preliminary study. As a control, samples of papaya and cucumber were sprayed using distilled water. Then, the fruits were dried and packed in a porous plastic bag. The samples were stored in a dark room (27 °C) until observations were made.

Fruit Color Measurement

Samples were observed using the Royal Horticultural Standard Color Chart (Sharmin et al., 2015). The level of ripeness of the papaya and cucumber was determined by matching the color fruit according to the color scale listed on the fruit color scale chart. Fruits were arranged from the smallest to the largest color scale, starting from green, breakers, turning pink, light red, and red. Furthermore, the fruits were photographed for documentation.

Total Soluble Solid (TSS) analysis

Measurement of TSS was carried out using a refractometer. The juices from the samples were dripped onto the refractometer glass. The refractometer was directed at the light to observe its value. The value was measured in three replications by taking the juices of the samples from several different sides of the material, namely the base, tip, and middle parts. The values obtained were expressed in °Brix units.

Vitamin C content analysis

The analysis started with the preparation of the samples. Fruit samples were mashed and measured for 5 g. Then the mashed samples were extracted with 25 ml of 80% acetone for 1 hour at room temperature. Samples were centrifuged at 4500 x g for 10 minutes. The obtained supernatant was used for the analysis. Vitamin C content was measured by titrating a 0.01 N iodine solution with starch as an indicator. 25 ml of fruit filtrate was titrated with 0.01 N iodine solution. The starch indicator was prepared by dissolving 1 g of starch into 100 ml of boiled distilled water. Before titration, the filtrate was added with a starch indicator. The occurrence of a blue color of Iod-starch indicates the end of the titration. The calculation of vitamin C with standardized Iodine was 1 ml of Iodine 0.01 N, equivalent to 0.88 mg of ascorbic acid.

Analysis of Total Phenol

The total phenol test used the Follin-Ciocalteu method, which measured absorbance at a wavelength of 765 nm (Viegas et al., 2016). 1 g of the sample was dissolved into 10 ml of distilled water. 0.5 ml of the solution was mixed with 5 ml of distilled water. This solution was mixed manually. After 5 minutes, the solution was mixed with 1.5 ml of 5% Na2CO3, added with 1.5 Folin-Ciocalteau reagent and shaken manually. After that, measurements were made with a spectrometer at a wavelength of 765 nm. Phenol content was determined based on the standard curve equation. The standard used for making the standard curve was gallic acid. Gallic acid standards were created with 5,10,20,30,40, and 50 ppm (Khadambi, 2007).

Total Antioxidant Activity (TAA) analysis

The samples were prepared as it was done in the total vitamin C content analysis. The obtained supernatant was used to measure the total phenol concentration and antioxidant activity of DPPH [7]. 4 mL of the supernatant was mixed with 4 mL of 50 ppm DPPH solution, shaken vigorously, and then incubated for 30 minutes at room temperature. After that, the absorption was measured using a spectrophotometer with a wavelength of 517 nm. Total antioxidant activity (TAA) was determined by the transfer method of 2,2-Diphenyl-1pircyl-hydrazine-hydrate (DPPH) (Kolniak-Ostek, 2016). The percentage of DPPH radical inhibition expresses TAA.

Statistical Analysis

Statistical analysis to determine the effect of abscisic acid on antioxidant content was carried out using Statistical Analysis System (SAS). All values were expressed in mean ± standard deviation. T-test analysis was used to determine the difference between treatments with a significance level of 5%.

RESULT AND DISCUSSION

Effect of Abscisic Acid on Fruit Color

Color is an important quality parameter for fruit ripening (Shen et al., 2019). Figure 1 and 2 show the changes in color in papaya and cucumber. Papaya treated with abscisic acid experienced a faster improvement in color than the control. This result is in line with the research of Wu et al. (2018), which reported that the external application of abscisic acid improved the color of tomato fruit. In this study, the application of abscisic acid in cucumber did not affect the speed of color improvement compared to the control. These results are similar to research by Wang et al. (2013), which reported that coating abscisic acid to cucumbers at the green ripening stage could not stimulate ripening.

Effect of Abscisic Acid on Total Soluble Solid

The application of abscisic acid in papaya significantly increased sugar accumulation on day

Figure 1. Effect of abscisic acid on the color of papaya fruit on (a) day 4 and (b) day 6

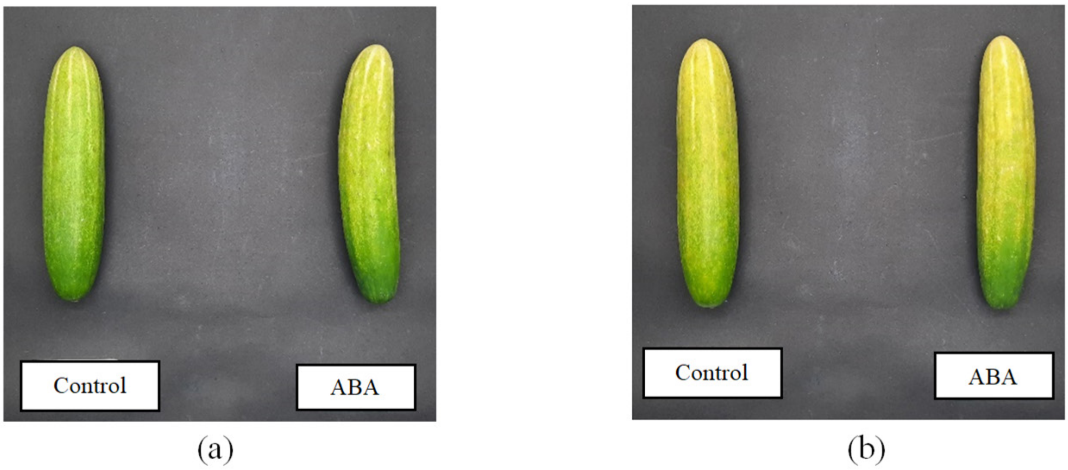


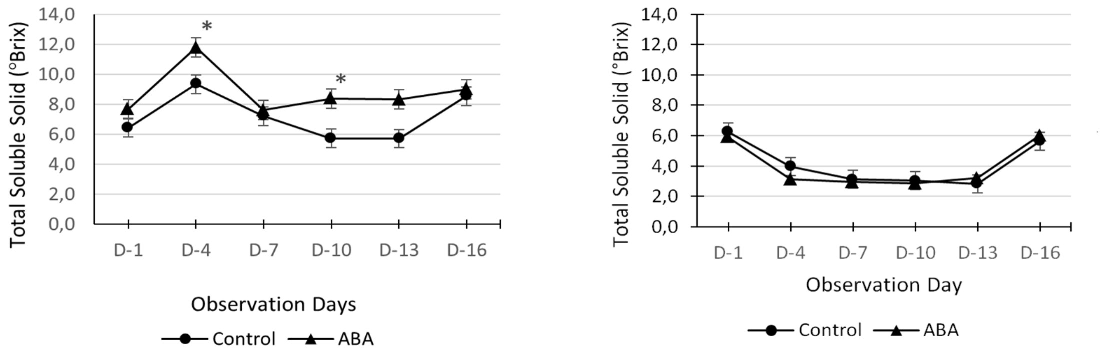
Figure 2. Effect of abscisic acid on color of cucumber fruit on (a) day 4 and (b) day 6

four and day ten compared to the control (Figure 3). The increase in sugar accumulation during fruit ripening showed that abscisic acid played a role in accelerating the starch breakdown into sugar during ripening, as reported by Zaharah et al.(2013). The results of this study are in line with the research of El-Mogy et al. (2019) on strawberries, which reported that abscisic acid was able to reduce the loss of total dissolved solids, and the research by (Luo et al., 2014) reported the same results on sweet cherry fruit.

The application of abscisic acid to cucumbers did not show a significant increase in total soluble solids compared to the control, which indicates that abscisic acid has fewer roles in the rate of sugar accumulation. This result is in line with the research of Wang et al. (2013), which reported that the application of abscisic acid to cucumbers at green stage maturity did not affect the development and maturity of the fruit.

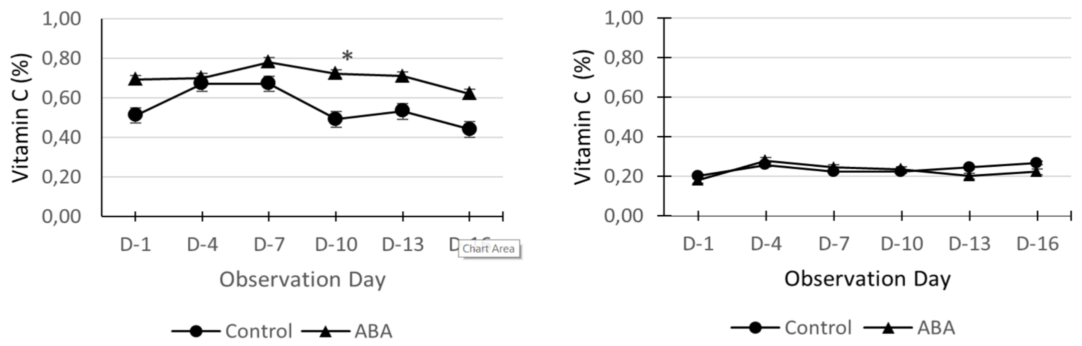
Effect of Abscisic Acid on Vitamin C Content

Vitamin C levels were increased in both control and treated subjects (Figure 4a). This increase continued until day seven, followed by a decline until the last observation day. Compared to control samples, applying ABA to papaya could significantly retain vitamin C levels until day ten storage, even though no significant difference can be seen on days 13 and 16. The effect of ABA on vitamin C



(a) (b)

Figure 3. Effect of abscisic acid on total soluble solids of (a) papaya and (b) cucumber



(a) (b)

Figure 4. Effect of abscisic acid on vitamin C of (a) papaya and (b) cucumber during storage

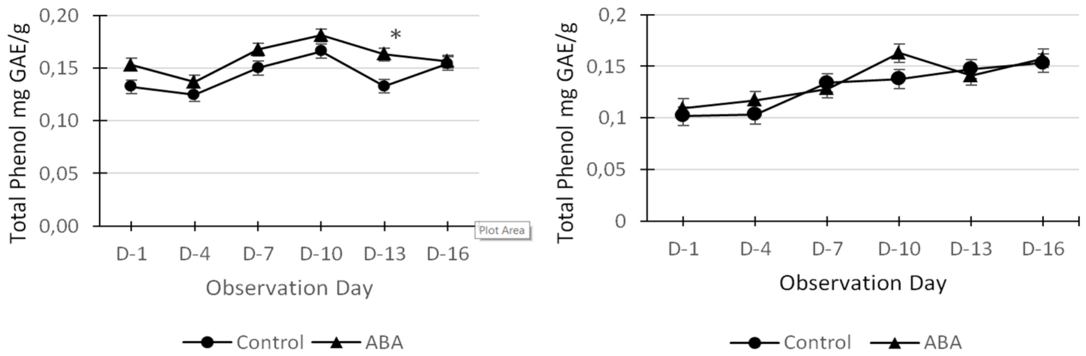
in this study is similar to a previous study by Tao et al. (2020) on tomatoes and Miret & Munné-Bosch (2016) on raspberries.

Vitamin C is a natural organic compound with antioxidant properties and multiple health benefits for humans (Pehlivan, 2017; Paciolla et al., 2019). However, a previous study reported that the effects of plant growth regulators such as ABA on the metabolism and content of vitamin C were limited (Lee & Kader, 2000). A previous study hypothesized the relationship between ABA and vitamin C content. Abscisic acid can induce cell wall degradation into pectin. Pectin contains galacturonic acid units, which contribute to the biosynthesis of vitamin C. Therefore, applying ABA to fruit may enhance vitamin C content (Miret & Munné-Bosch, 2016).

In Figure 4b, the application of ABA on cucumber, including a non-climacteric fruit, showed no significant effect on vitamin C compared to the control. Vitamin C content in cucumbers peaked on day four and decreased until the end of observation days. This decline was due to oxidation, as seen in a previous study by Tijero et al. (2016) on another example of non-climacteric fruit, the sweet cherry.

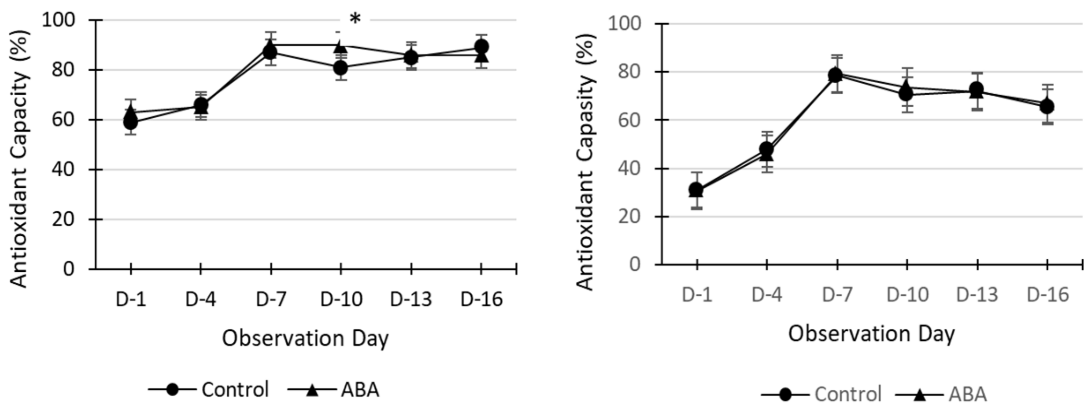
Effect of Abscisic Acid on Total Phenol

The effect of abscisic acid on changes in total phenol content during the ripening of papaya and cucumber can be seen in Figure 5. The total phenol content in the control papaya fruit increased dur-



(a) (b)

Figure 5. Effect of abscisic acid on total phenolic content of (a) papaya and (b) cucumber



(a) (b)

Figure 6. Effect of abscisic acid on antioxidant capacity of papaya (a) and (b) cucumber

ing day 4 of observations. It reached the maximum on day ten of observations, followed by a decrease until the end of the observation. The same increase was seen on papaya treated with abscisic acid, as its total phenol content significantly differed from the control sample on day 13. The application of abscisic acid in cucumbers increased the total phenol content from day 4 to day 10. However, the abscisic acid treatment did not show a significant increase in the total amount of phenol compared to the control during storage.

Phenol compounds are a group of bioactive molecules scattered in various plant types. The level of phenol compounds in plants is affected by many factors, such as environmental stresses and exogenous elicitors (Pérez-Balibrea et al., 2011). Abscisic acid is a phytohormone that functions as a natural elicitor that can influence the content of phenolic compounds depending on plant varieties. The main phenolic compounds in papaya include ferulic acid, caffeic acid, and coumaric acid (Kadiri et al., 2017). In this study, the application of abscisic acid in papaya significantly increased the total phenol content compared to the control. These results are in line with the results of previous studies on tomatoes (Tao et al., 2020; Wu et al., 2018), sweet cherries (Luo et al., 2014), and strawberries (Siebeneichler et al., 2020). On the other hand, abscisic acid did not result in the phenol content of cucumber during storage. This result is similar to other abscisic acid applications in the non-climacteric fruit of the Muscardine grape (Sandhu et al., 2011) and blueberries (Buran et al., 2012).

Effect of Abscisic Acid on Antioxidant Capacity

The antioxidant capacity of papaya and cucumber was evaluated using the DPPH radical scavenging activity method. The effect of abscisic acid on antioxidant capacity showed that the antioxidant capacity of papaya fruit was significantly higher on day 10 of observation compared to the control (Figure 6). The antioxidant capacity increased by 11.1% on day 10 of observation compared to the control.

The antioxidant capacity of cucumbers on days 4 and 7 also showed an increase; however, the application of abscisic acid did not show a significant difference with the control. According to Sandhu et al. (2011), the external application of abscisic acid can intensify plant responses to develop and increase phenolic compounds biosynthesis in fruit and other plant organs. The present study showed a significant increase of antioxidant capacity in papaya treated with ABA compared to the control. This increase coincided with an increase in total phenol content, indicating that phenolic compounds are the main contributors to antioxidants in papaya fruit. Thus, the results of this study indicate that abscisic acid resulted in higher antioxidant capacity through phenolic compound accumulation during the ripening stage of papaya fruit.

Applying abscisic acid to cucumbers did not produce any difference in antioxidant capacity compared to the control. This result was consistent with abscisic acid's effect on phenolic compounds' content, which showed no significant difference between the cucumbers treated with abscisic acid and the control. The same results were reported on blueberries (Buran et al., 2012) and grapes (Giribaldi et al., 2010).

According to Buran et al. (2012), abscisic acid increases the speed of climacteric fruit ripening by increasing ethylene synthesis or fruit sensitivity to ethylene. However, the effect of abscisic acid on non-climacteric fruit ripening is primarily determined by the type and variety. It was also reported that abscisic acid did not affect the total phenol content in ripe berries and bush blueberries. The administration of abscisic acid did not affect the antioxidant activity of ripe blueberries, which were analyzed using the ORAC method.

CONCLUSION

External application of abscisic acid increased the content of bioactive compounds and maintained postharvest quality in papaya fruit (climacteric) during ripening. In contrast, the application of abscisic acid with a concentration in cucumber (non-climacteric) showed a content of bioactive compounds and quality that did not differ significantly from the control.

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