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EVALUATION OF ANTIOXIDANT PROPERTIES OF *Cucumis melo* L cv. Hikapel DURING STORAGE AT ROOM TEMPERATURE

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ABSTRACT

The changes in antioxidant compounds and antioxidant activities of melon (*Cucumis melo* L.) cultivar Hikapel during postharvest storage at room temperature were evaluated. Melon with three ripening stages (27 DAA, 29 DAA, and 32 DAA) were harvested and stored at 25^{o}C for 20 days. Melon cv. Hikapel were evaluated for their antioxidant compounds such as ascorbic acid, total phenolic (TPC), and total flavonoid content (TFC). Antioxidant capacity was also evaluated using DPPH radical scavenging assay (DPPH-RSA) and ferric reducing power assay (FRPA). The result showed that there were different levels of antioxidant compounds (TPC, TFC, and AAC) and antioxidant activities (DPPH-RSA & FRPA) in different ripening stages of this melon. Antioxidant compounds and antioxidant activity decreased during postharvest storage. In conclusion, melon cv. Hikapel provides various natural antioxidant compounds such as phenolic, flavonoid which can be the main contributors to the overall antioxidant activity of melon cv. Hikapel and its antioxidant properties were influenced by the postharvest storage period.

Keywords: *Cucumis melo* L. cv. Hikapel, antioxidant, ripening stage, room storage

INTRODUCTION

Cucumis melo L. cv. Hikapel, a new cultivar of melon, had been developed in the Laboratory of Genetic and Breeding, Faculty of Biology, Universitas Gadjah Mada (UGM), Indonesia. The melon cv. Hikapel is one of the non-netted orange-fleshed melons. This melon has several quality values, such as an orange-fleshed, sweet taste, crunchy texture, and good shelf life. This melon has been known for its antioxidant properties, such as ascorbic acid, carotenoid, phenolics, and flavonoid (Wulandari *et al.*, 2017). These compounds are suggested to be major bioactive compounds for health benefit. Nowadays, the consumption of fruits has been associated with reduced risk of chronic diseases. The awareness about the importance of nutritional requirements among consumers has increased due to people's healthy lifestyle.

It has been reported that the levels of health-promoting bioactive compounds of fruits are strongly influenced by the ripening stage and postharvest storage time (Arancibia-Avila *et al.*, 2008; Zainuddin *et al.*, 2014; Zhang *et al.*, 2008). The study of changes in bioactive compounds during ripening of fruit and during storage time has great relevance both to human health and commercial purposes. That study provides valuable information to understand the alteration of those compounds and to evaluate the best harvesting time and postharvest storage time to get the highest antioxidant potential. Harvesting time is essential to get high-quality fruit with good nutrition potential. Postharvest storage time is crucial to determined how long fruit can be stored at room temperature storage.

As a new melon cultivar, the study of antioxidant potential in this fruit is still rare. The study focused on antioxidant activity

alteration during postharvest storage of melon cv. Hikapel has never been done. Therefore, this research aimed to study the changes in antioxidant activity and antioxidant compounds during postharvest storage at room temperature.

MATERIALS AND METHODS

Plant Materials and Chemicals

Melon (*Cucumis melo* L.) cv. Hikapel was cultivated in Agricultural Training, Research and Development Station of Universitas Gadjah Mada (KP4 UGM), Desa Kalitirto, Berbah Sub-District, Sleman Regency of the Special Region of Yogyakarta. Melon with three ripening stages S1 (27 days after anthesis (DAA)), S2 (29 days after anthesis (DAA), and S3 (32 days after anthesis (DAA)) was harvested and transferred to the laboratory immediately.

The melon was washed with NaOCl 50 ppm, rinsed with water, air dried, and stored for 20 days at room temperature. Melon was cut, deep freeze, and freeze-dried. The freeze-dried samples were ground and extracted using methanol: acetone (80:20) acidified with acetic acid, sonicated and centrifuged at 4000 g for 10 min at 4ºC. The supernatant was used for further analysis such as total phenolic, total flavonoid, DPPH-RSA, and FRP assay.

All chemicals used in this experiment have a pro analysis grade. H3PO4, AlCl3.6H2O, FeCl3.6H2O, Na2CO3, K3Fe(CN)6, methanol, ethanol, acetone, petroleum ether, acetic acid were purchased from Merck. Folin-Ciocalteu phenol reagent, TCA, 2,2-dipyridyl, quercetin, gallic acid, ascorbic acid, 2,2-diphenyl–1-picrylhydrazyl radical (DPPH).

Methods

Total phenolic content (TPC) determination

The amount of total phenolic content (TPC) in melon extracts was determined according to the Folin–Ciocalteu method (Dewanto *et al*., 2002). Samples 125 µL were introduced into test tubes in which containing 125 µL of Folin–Ciocalteu's reagent and 250 µL of sodium carbonate (7.5%, w/v), mixed by vortex and allowed to stand in darkness at room temperature for 90 min. The absorbance was measured spectrometrically at 760 nm. The total phenolic content was expressed as mg gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g DW). All measurements were done in triplicate.

Total flavonoid content (TFC) determination

Total flavonoid content was estimated according to the procedure of Santas *et al.* (2008) based on the aluminum chloride complex formation. To 1 mL of supernatant added with 1 mL of 2% (w/v) $AlCl_3$ methanolic solution. The mixture was then allowed to react for 2 min at room temperature and the absorbance was read at 410 nm. Total flavonoid content was calculated as mg quercetin equivalent per 100 g of fresh weight (mg QE/100 g DW). All measurements were done in triplicate.

Ascorbic acid content (AAC) determination

Ascorbic acid content was determined by the 2,2-dipyridyl method (Giudice *et al.*, 2015). This method is based on the reduction of Fe^{3+} to Fe^{2+} by ascorbic acid and detection of Fe^{2+} complexed with 2,2-dipyridyl. Five grams of frozen flesh were homogenized in 5 mL of5% (w/v) trichloroacetic acid (TCA). The homogenate was filtered and centrifuged for 10 min at 12,000 g (4ºC). Then, 20 µL of the supernatant mixed with 20 µL 0,4 M phosphate buffer (pH 7.4) and 10 µL distilled water.

Eighty microlitres of colour reagent solution, prepared by mixing solution 1 (31% H_3PO_4, 4.6% (w/v) TCA, and 0.6% (w/v) $FeCl_3$) with solution 2 (4% 2,2′-dipyridil (w/v) made up in 70% ethanol) at a proportion of 2.75:1 (v/v), were added. The mixture was incubated at 37 °C for 40 min, then cooled down immediately to room temperature. Absorbance was read at 525 nm. Ascorbic acid content was expressed in mg AA/100 g DW. All measurements were done in triplicate.

Ferric reducing power assay (FRPA)

The ferric reducing power of the melon fruit extracts was performed by using the potassium ferricyanide–ferric chloride method (Berker *et al.*, 2007). One mL of extract was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide ($K_3Fe(CN)_6$). The mixtures were incubated at 50ºC for 20 min, and 2.5 mL of 10% (w/ v) trichloroacetic acid (TCA) was added. 2.5 mL of the mixture was taken and mixed thoroughly with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ (w/v). The

absorbance of the blue green color was measured at 700 nm. Ferric reducing power activity was expressed as mg ascorbic acid equivalents per 100 g of fresh weight (mg AAE/100 g DW). All measurements were done in triplicate.

DPPH radical scavenging activity assay (DPPH-RSA)

The DPPH radical-scavenging activity of the melon fruit extracts was estimated as described by Sharma *et al*. (2009). Briefly, 0.3 mL sample extract was mixed with 2.7 mL of 100 μM DPPH in a methanolic solution. The mixtures were left for 40 min in the darkness at room temperature, the absorbance was then measured at 517 nm. All measurements were done in triplicate. The percentage of DPPH radical-scavenging activity was calculated using the following equation:

$$\% \text{ RSA} = [(\text{Abs. Blank} - \text{Abs. Sample})/(\text{Abs. Blank})] \times 100\%$$

Statistical analysis

The effects of postharvest storage time on the antioxidant properties of melon cv. Hikapel at different ripening stages were analyzed by one-way analysis of variance (ANOVA). Tuckey test was carried out to identify significant differences between ripening stages and postharvest storage time. Mean values with $p<0.05$ were considered statistically significant. For analysis of the correlation between antioxidant capacity and antioxidant compounds, the pearson correlation was carried out. All statistical analysis was performed using SPSS 20.

RESULTS AND DISCUSSION

Cucumis melo L cv Hikapel at ripening stage S3 can only be stored until the 10th day of storage. *Cucumis melo* L cv Hikapel at ripening stage S1 can be stored until 20th and ripening stage S2 can be stored until the 15th day of storage. Melon cv. Hikapel provides wide variety of antioxidant compounds such as carotenoid, phenolic, flavonoid, and ascorbic acid. The statistical analysis revealed that TPC decreased significantly during the on tree-ripening.

Total phenolic content reached the highest concentration at ripening stage S1 (124.75 ±13 GAE/100 g DW) and decreased to 92.20±04 mg GAE/100 g DW (S3). Similarly, significantly decreased in total phenolic as in cantaloupe melon (Abu-Goukh *et al*., 2011). There was no chance of total flavonoid content during ripening of melon cv. Hikapel. Ascorbic acid content and total carotenoid content increased during ripening stages. Ascorbic acid content reached the highest value at ripening stage S3 (243.92 ±11 mg AA/100 g DW). It showed that ascorbic acid was synthesized during on-tree ripening of melon cv. Hikapel. These results are in agreement with several studies reported in teasel gourd (Singh *et al*., 2015) and carambola fruit (Zainuddin *et al*., 2014).

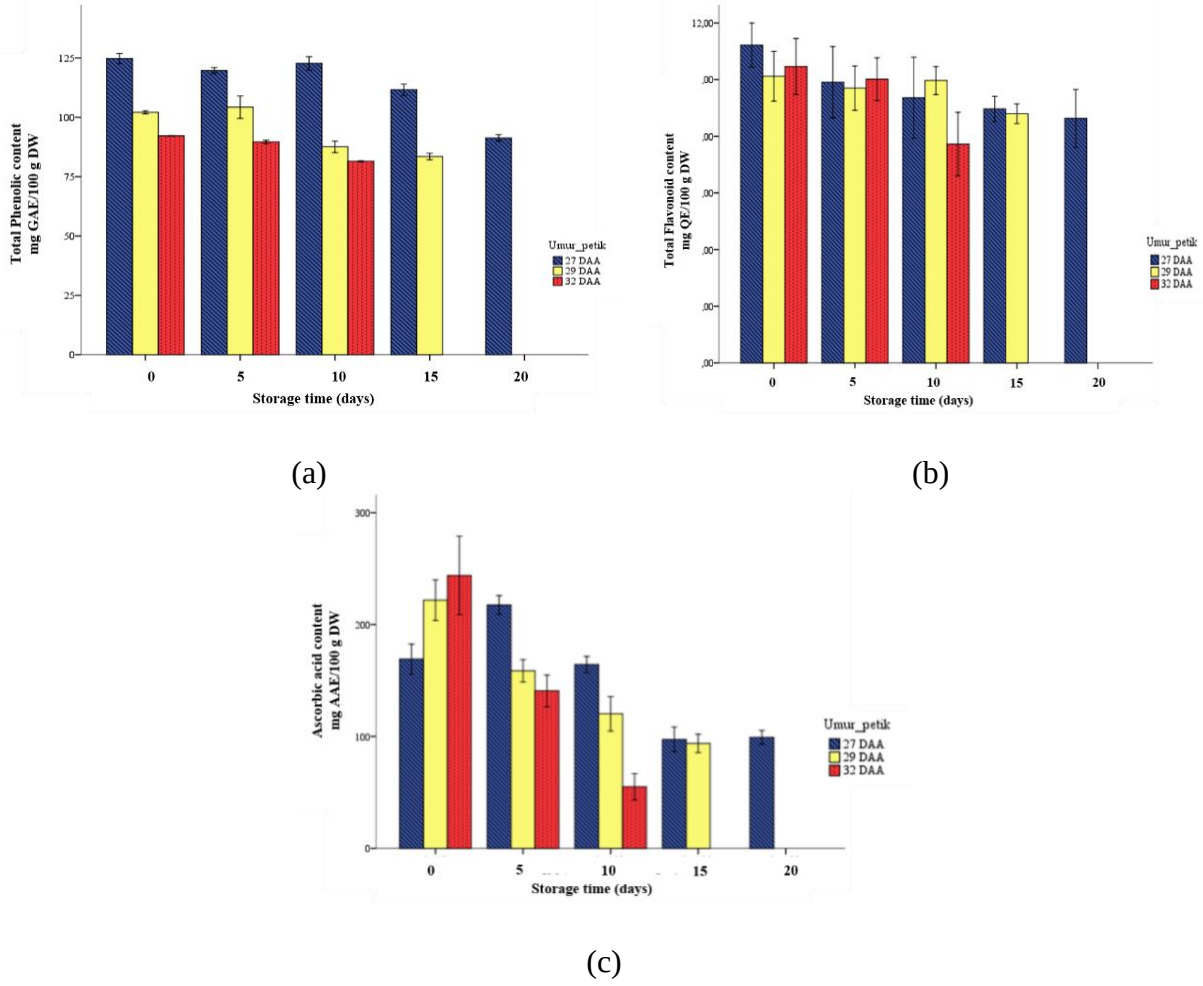
Total -phenolic, -flavonoid, - ascorbic acid content of melon cv. Hikapel were changed during postharvest storage at room temperature as presented in Fig. 1. Total -phenolic and –flavonoid, ascorbic acid content were decreased during postharvest storage. Ascorbic acid content decreased during storage might be related to the increased of *ascorbate peroxidase* activity during early storage time. But when the *ascorbate peroxidase* starts to decrease in their activity, the ascorbic acid content still decreased. It might be caused by the use of ascorbic acid for oxalate or tartrate synthesis.

Ascorbic acid catabolism occurs with tartrate and oxalate as major products (Smirnoff and Pallanca, 1996). Therefore, it is also possible that ascorbic acid in melon cv. Hikapel is being driven to other routes, such as oxalate and tartrate production, such as in guava and mango (Gomez and Lajolo, 2008). Generally, when fruits become over-ripe, vitamin C content declines, concurrently with the degradation of fruit tissues (Kalt, 2005).

Total -phenolic, -flavonoid content decreased during fruit storage can be related to the expression of phenolic biosynthetic enzyme genes, *phenylalanine ammonia*-lyase (PAL) and the phenolic oxidizing enzyme, *polyphenol oxidase* (PPO) and *peroxidase* (POX). Increasing in PPO activity and decreasing PAL activity was also the possible contribution to the TPC and TFC decreasing value as reported in carambola fruit (Zainuddin *et al.*, 2014).

Another possible reason for the decrease in ascorbic acid, phenolic, and flavonoid content might be due to the decrease of internal

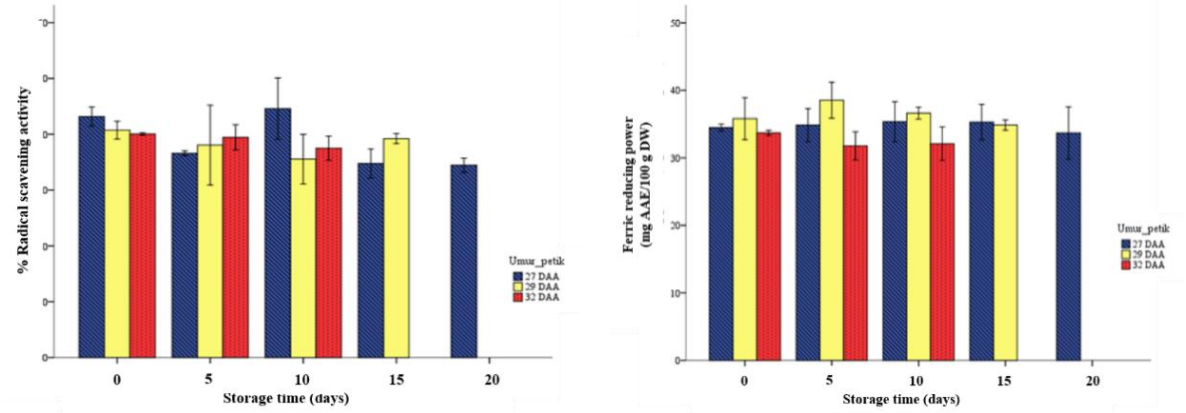
antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), APX, and guaiacol peroxidase (G-POD) making the cells turn to other reserved antioxidant compounds such as phenolic, flavonoid, and ascorbic acid in maintaining cellular integrity (Huang *et al.*, 2007).



(a) (b)

(c)

Figure 1. Total Phenolic Content (a), Total Flavonoid content (b), Ascorbic acid content (c) of *Cucumis melo* L. cv. Hikapel during postharvest storage at room temperature.



(a) (b)

Figure 2. DPPH radical scavenging activity (a), Ferric Reducing Power (b) of *Cucumis melo* L. cv. Hikapel during postharvest storage at room temperature

Based on the DPPH-RSA the fruit of all ripening stages was capable to scavenge free radicals via the hydrogen-donating mechanism. All samples indicated high

antioxidant potential as strong as Vitamin C at a concentration of 50 ppm. The level of DPPH radical scavenging activity decreased significantly up to S2 and stable to the end-stage (S3). The ferric reducing power may serve as a significant indicator of the antioxidant potential. This assay evaluates the ability of the extracts to reduce Fe^{3+} to Fe_{2+}, recorded as Perl's Prussian blue formation. All ripening stages of melon cv. Hikapel showed reducing power activity, which was more than 30 mg AA equivalent. The reducing power of the fruits was not significantly different among S1 and S2; S1 and S3.

In general, the antioxidant capacity of melon cv. Hikapel also decreased during on-tree ripening due to a decrease of the total -phenolic, -flavonoid, ascorbic acid content as observed in carambola (Zainuddin *et al*., 2014) and durian (Arancibia-avila *et al*., 2008). It is known that ascorbic acid and phenolic compounds, especially flavonoid compounds responsible for free radical scavenging. However, antioxidant activity does not only depend on phenolic concentration itself but also depend on any other compounds such as ascorbic acid, flavonoid, and carotenoid (Tavarini *et al*., 2008; Gardner *et al*., 2000).

Therefore, the antioxidant activities in fruit cannot be attributed solely to their phenolic contents, but also to the actions of different antioxidant compounds present in the fruits. There was a correlation between antioxidant activity and antioxidant contents of melon cv. Hikapel (data not shown). Antioxidant activity (DPPH-RSA, FRPA) was positively correlated with total phenolic, total flavonoid, and ascorbic acid content. It is also known that fruits with high antioxidant capacity generally contain more antioxidants and most of these antioxidants has been showing to be phenolic compounds and in particular flavonoid.

CONCLUSION

All ripening stages of melon cv. Hikapel provides various dietary antioxidants such as phenolics, flavonoids, and ascorbic acid and showed free radical scavenging and reducing power activity. There were different levels of ascorbic acid, total phenolic, and total flavonoid -content in different ripening stages of this melon. Antioxidant compounds and antioxidant activity decreased during storage at room temperature. At the end of storage time, S1 has the highest antioxidant compound and capacity, whereas S3 has the lowest antioxidant compound and capacity. In conclusion, the ripening stage and postharvest storage time are important factors on the antioxidant properties of this melon cultivar Hikapel. Therefore, the grower should determine the appropriate harvesting time and postharvest storage duration at room temperature to meet commercial purposes with high antioxidant potential.

REFERENCES

Abu-Goukh, AA, BarakaAl FM, and Elballa MMA. 2011. Physico-chemical changes during growth and development of 'Galia' cantaloupes. II. Chemical changes. *Agriculture Biology Journal North America*. 2 (6): 952–963

Arancibia-Avila P, Toledo F, Park Y S, Jung ST, Kang SG, Heo BG.2008. Antioxidant properties of durian fruit as influenced by ripening. *LWT-Food Science and Technology*. 41: 2118-2125.

Berker K I, Güçlü K, Tor I, dan Apak R. 2007. Comparative evaluation of Fe(III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. *Talanta*. 72 :3: 1157–65

Dewanto V, Wu XZ, Adom KK., Liu RH. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*. 50: 3010–3014.

Gardner HW. 1995. Biological roles and biochemistry of lipoxygenase pathway. *HortScience* 30: 197–204.

Giudice RD, Raiola A, Tenore GC, Frusciante L,Barone A, Monti DM, Rigano MM. 2015. Antioxidant bioactive compounds in tomato fruits at different ripening stages and their effects on normal and cancer cells. *Journal Functional Foods*. 18: 83–94.

Huang R, Xia R, Hu L, Lu Y, Wang M. 2007. Antioxidant activity and oxygen-scavenging system in orange pulp during

fruit ripening and maturation. *Scientia Horticulturae*. 113: 166–172

Kalt W, Forney CF, Martin A, Prior RL. 1999. Antioxidant Capacity, Vitamin C, Phenolics, and Anthocyanins after Fresh Storage of Small Fruits. *Journal of Agricultural and Food Chemistry*. 47 (11) : 4638–4644.

Liu D, Shi J, Ibarra AC, Kakuda Y, Xue SJ. 2008. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and β-carotene mixtures on the DPPH free radical. *Food Science and Technology*. 41: 1344–1349.

Müller L, FröhlichK, BöhmV. 2011. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (a TEAC), DPPH assay and peroxyl radical scavenging assay. *Food Chemistry*. 129: 139–148.

Perkins-Veazie P, Collins JK. 2006.Carotenoid Changes of Intact Watermelons after Storage. *Journal of Agricultural and Food Chemistry*. 54 : 5868−5874.

Santas J, Carbo R, Gordon M, Almajano M. 2008. Comparison of the antioxidant activity of two Spanish onion varieties. *Food Chemistry*. 107 :1210–1216.

Sharma OP, Bath TK. 2009. Analytical Methods DPPH antioxidant assay revisited. *Food Chemistry*. 113 :1202–1205.

Singh S, Swain S, Nishac M, Banu VS, Singh DR, Roy SD. 2015. Changes in lycopene, total carotenoid and anti-radical activity in teasel gourd [Momordica subangulata ssp. renigera (G. Don) de Wilde] fruit fractions at different stages of maturity. *Industrial Crops and Products.* 73 154–163.

TavariniS, Degl'Innocenti E, Remorini D, Massai R, Guidi L. 2008. Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. *Food Chemistry*. 107: 282–288.

Thaipong K,Boonprakoba U, Crosby K., Cisneros-Zevallosc L, Byrne DH. 2006. Comparison of ABTS,DPPH,FRAP, andORACassays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition Analysis*. 19: 669–675.

Wulandari P, Daryono BS, Supriyadi. 2017. The Effect of Ripening Stages on the Antioxidant Potential of Cucumis melo L cv. Hikapel. *AIP Conference Proceedings* 1854: 020039

Zainudin MAM, Hamid AA, Anwar F, and Osman A. 2014. Variation of bioactive compounds and antioxidant activity of carambola (Averrhoa carambola L.) fruit at different ripening stages. *Scientia Horticulturae*. 172: 325–331.

Zhang W, Li X, Zheng J, Wang G, SunC., Ferguson I., Chen K. 2008.Bioactive components and antioxidant capacity of Chinese bayberry (Myrica rubra Sieb. and Zucc.) fruit in relation to fruit maturity and postharvest storage. *European Food Research and Technology*. 227:1091- 1097.