Indonesian Journal of Biotechnology

VOLUME 24(2), 2019, 88-93 | RESEARCH ARTICLE

Analysis of ethylene biosynthesis gene expression profile during titanium dioxide (TiO_2) treatment to develop a new banana postharvest technology

Fenny M. Dwivany[1,2,3,4,*] Rizkita R. Esyanti[1,3], Veinardi Suendo[5,6], Aksarani 'Sa Pratiwi[1], Annisa A. Putri[1,3,4]

[1]School of Life Science and Technology, Institut Teknologi Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia
[2]ForMIND Institute, Bandung 40132, Indonesia
[3]Bali International Research Center for Banana, Universitas Udayana, Bukit Jimbaran, Kuta Selatan, Badung, Bali 80361, Indonesia
[4]Bioscience and Biotechnology Research Center, Institut Teknologi Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia
[5]Chemistry Department, Institut Teknologi Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia
[6]Research Center for Nanoscience and Nanotechnology, Institut Teknologi Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia
*Corresponding author: fenny@sith.itb.ac.id

SUBMITTED 17 Juny 2019 **REVISED** 23 August 2019 **ACCEPTED** 4 December 2019

ABSTRACT Banana is an important crop that demands proper methods in postharvest handling. As a climacteric fruit, the banana fruit ripening process is affected by ethylene. Several methods have been developed to extend the shelf life of a banana, such as using ethylene scrubbers. In this study, titanium dioxide (TiO_2), a photocatalyst, was used as an alternative method to delay the fruit ripening process. The effect of TiO_2 on the ripening-related gene *MaACS1* was investigated. Banana fruits were placed in a TiO_2-coated glass chamber and observed for ten days. Fruit ripening in the treated chamber was delayed for eight days compared to the control. Total RNA was extracted from control and TiO_2-treated fruit pulp and synthesized into cDNA. Reverse transcription PCR was performed to investigate the gene expression, which showed that *MaACS*1 expression was relatively lower than treated control. The finding of these studies suggested that the TiO_2 chamber has the potential to extend the shelf life of banana by delaying its ripening process and decreasing the expression of *MaACS1*. To the best of our knowledge, no previous study has investigated the effect of TiO_2 on the expression of genes related to banana fruit ripening.

KEYWORDS banana; ripening; TiO_2; postharvest; gene expression

1. Introduction

Banana is one of the most consumed fruits and categorized as an important commodity in various countries. The highly nutritious contents in banana such as vitamins A, B1, B2, and C make the fruit popular around the world (Cano et al. 1997). Indonesia is one of the top 10 countries in banana production (FAO 2019). However, Indonesia also faces problems in the postharvest handling of banana production. The majority of postharvest handling of banana is performed traditionally, which consequently leads to low-quality banana production. Some of the banana agroindustries around the world have attempted using a modified atmosphere and a controlled atmosphere technology to extend the shelf life of the fruit. However, such techniques may not be effectively applicable, especially for small Indonesian farmers in rural areas. Therefore, there is a need for other alternatives such as low energy technologies to extend the shelf life of banana by delaying its ripening process.

Ripening is a natural process that occurs in fruits and is highly influenced by a gaseous phytohormone, ethylene (C_2H_4) (Guo and Ecker 2004). Ethylene is synthesized from S-adenosyl methinonine (AdoMet), which is then converted into 1-aminocyclopropane-1 carboxylic acid (ACC) with the help of the enzyme ACC synthase (Yang and Hoffman 1984). ACC is the intermediate precursor to generate ethylene. ACC is then converted into ethylene with the help of an ACC oxydase enzyme. Studies have demonstrated that *MaACS*1, a member of the ACC synthase (*ACS*) gene family, is responsible for the ripening process of the fruit (Liu et al. 1999; Karmawan et al. 2009; Dwivany et al. 2016).

Titanium dioxide (TiO_2) has been used as a photocatalyst to degrade ethylene that is emitted by fruits at low temperatures (Hussain et al. 2010). Another recent study conducted using TiO_2 nanofiber has demonstrated a delay in banana softening and color change (Zhu et al. 2019). It has been reported that ethylene degradation by TiO_2 produces carbon-dioxide and water and triggers a low ratio of O_2 to CO_2 in the atmosphere (Charoenshap et al. 2012). These conditions result in the reduction of ethylene biosynthe-

sis since oxygen is needed to convert ACC to ethylene by ACC oxidase (Kanellis et al. 2009). As mentioned above, ACC synthase is also an important enzyme which its activity also triggered by ethylene auto-catalytic reaction. Thus, lower ethylene production will influence this reaction (Inaba et al. 2007). In the present study, we investigated the effect of TiO_2 applied as a thin coating on the process of fruit ripening and the expression of the banana ripening-related ACC synthase gene, *MaACS1*. The results of this study demonstrated that TiO_2 has the potential to extend the shelf life of banana fruit.

2. Materials and Methods

2.1. Fruit Storage Chamber Preparation

Fruit storage chambers made from glass (1.5 L) were used in the experiment. The chambers were coated with a thin layer of TiO_2 that was prepared by mixing crystal TiO_2 (J25) nanoparticles in ethanol as a solvent. The anatase crystal had a pH range of 3.5–4.5. The TiO_2 nanoparticles were coated on the chambers using an airbrush sprayer and then heated using a shot gun. All preparations were performed at the Chemistry Department, Institut Teknologi Bandung. Charcoal pouches were placed in all chambers to absorb water produced from respiration.

Best quality Cavendish bananas were assorted, placed inside the chambers, and stored until ripening for approximately ten days. The observation time points were chosen from day 0 to day 8 (D-day, D-1, D-3, D-6, and D-8). Control group bananas were placed in a closed chamber without light exposure, whereas treated group bananas were placed in a closed chamber with UV radiation (300–400 nm) for 24 h. Both treatments were performed at room temperature (26°C–27°C). The observation was performed at Bioscience and Biotechnology Research Center, Institut Teknologi Bandung.

2.2. Physical and Physiological Analysis

Physical and physiological characteristics of the bananas were evaluated based on peel color changes, starch content measurements using the iodine test, pulp-to-peel ratio measurements, and sugar content values measured using total soluble solids (TSSs) (Dadzie and Orchard 1997). Peel discoloration and starch content measurements based on the iodine test during ripening were documented using a Canon IXUS 230 HS digital camera. Meanwhile, TSS measurements were performed using a hand refractometer (Atago™).

2.3. Gene Expression Profile Analysis

Molecular analysis was conducted to investigate the pattern *of MaACS1* gene expression in control and treated groups using gene-specific primers (Karmawan et al. 2009; Handayani and Dwivany 2014), wherein *MaGAPDH* was used as a reference gene to compare the expression profile. Total RNA was extracted as described by Cordeiro et al. (2008) with some modifications. The total RNA was then synthesized into complementary DNA (cDNA) using iScript™ cDNA Synthesis (Biorad catalog number: 170-8890). Gene expression profile was analyzed by reverse transcription PCR (RT-PCR) using the GreenTaq Master Mix kit (Promega, catalog number: M7122). All the PCR results were confirmed by electrophoresis on 1% (w/v) agarose. Semiquantitative analysis of gene expression was performed using ImageJ quantification (http://www.imagej.net) as well as by T-test analysis.

3. Results and Discussion

3.1. Physical and Physiological Analysis

Before conducting molecular analysis, the physical and physiological characteristics of all bananas from the control and treatment groups were evaluated (Dadzie and Orchard 1997). Based on the ripening stage, the fruit can be divided into two categories, climacteric, and nonclimacteric (Friend and Rhodes 1981). Banana is a climacteric fruit and has the unique feature of ethylene production during its ripening stage. During the entire ripening process, the cells undergo several physical and physiological as well as molecular changes. Banana peel color changes can be easily monitored by physical analysis before conducting physiological and molecular analyses.

The assessments in this study included observation of color changes in the banana peel, starch content analysis, pulp-to-peel ratio measurement, and TSS analysis. Color changes in the peel in both the control and treatment groups were observed during the ripening stage (Figure 1). Data were collected at the observation points D-day, D-1, D-3, D-6, and D-8. Results showed that treatment using the TiO_2 chamber could delay the ripening process as indicated by the color changes from mature green to yellow and brown. Banana peels in the control group were generally brown in color since day 6, whereas the treated banana peels were green to yellow in color.

Regarding starch content analysis, Dadzie and Orchard (1997) had stated that this method is simple, rapid, and inexpensive to detect starch conversion into sugar during the ripening process visually. In this study, the treated bananas showed differences compared with the control group bananas, and after six days of observation, the pulp still had the black coloration (Figure 1). This indicated that the treated group had abundant starch content compared with the control group. This result was also confirmed by the TSS values (Figure 2). Bananas from both the control and treatment groups exhibited an increment in the ^{o}Brix during ripening. The faster ripening process in the control group was also confirmed by its ^{o}Brix value that was higher from D-day to D-6 than that in the treated group. However, on D-8, the sugar content in the control group was found to be lower than that in the treatment group. Regarding the color changes in the banana peel from green to yellow and brown after being overripened, studies have reported that these changes are caused by

FIGURE 1 Color changes during ripening and the starch content analysis from control group [A] and treated group (exposed with UV light and stored in FSC) [B] from each day of observation. The observation points were choosen between the day of observation to day 8 (D-day, D-1, D-3, D-6, D-8).

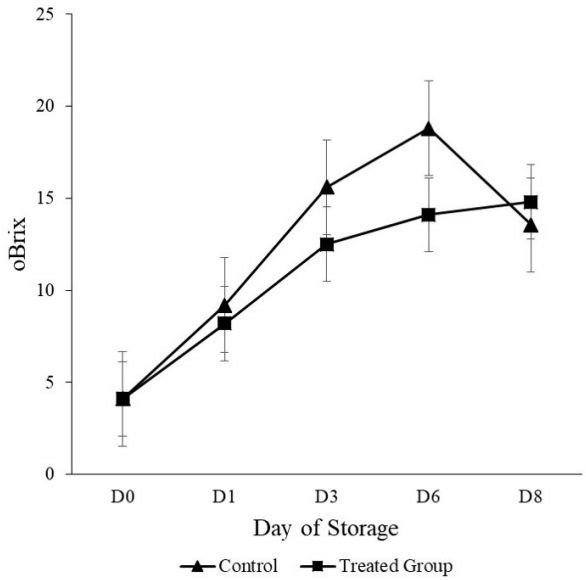

FIGURE 2 Total soluble solids of control and treated group from the pulp juice. The observation points were choosen between the day of observation to day 8 (D-day, D-1, D-3, D-6, D-8).

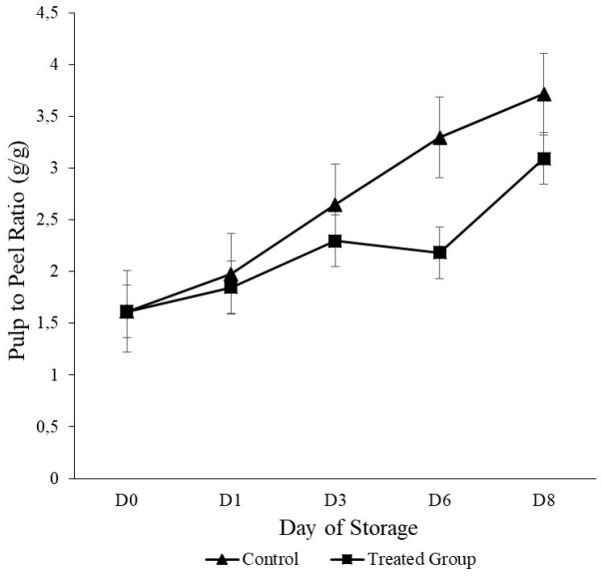

FIGURE 3 Pulp to peel ratio from the control and treated groups. The observation points were choosen between the day of observation to day 8 (D-day, D-1, D-3, D-6, D-8).

the degradation of chlorophyll by the chlorophyllase enzyme (Matile et al. 1996; Duan et al. 2007). Furthermore, the yellow coloration on the peel is due to the increasing amount of carotenoid pigments in the fruit (Subagio et al. 1996). The brown spot or senescent spotting that appeared on the ripe banana could be due to cell necrosis as a result of chlorophyll degradation (Mosera et al. 2009). The color and size of the spot could increase rapidly during the ripen-

ing process (Karmawan et al. 2009). In addition, it has been reported that starch contributes to 20% of the major component in the fruit and gets converted into a carbon source during ripening (Bierhals et al. 2004). During the process of ripening, starch conversion results in a sweeter fruit. In this study, the starch content was assessed qualitatively by the iodine test, in which iodine reacts with starch and results in black color around the fruit flesh. On the other hand, the sugar content analysis done by measuring the TSS in the pulp of the fruit indicated that TSS values increased as the ripening process progressed (Dadzie and Orchard 1997).

These results also corresponded to the pulp-to-peel ratio measurement. Based on the data obtained, both the control and treatment groups exhibited an increase in the pulp-to-peel ratio during the ripening process (Figure 3). However, the pulp-to-peel ratio has been considered to be constant and better in assessing the ripening index (Dadzie and Orchard 1997). A change in the pulp-to-peel ratio is one of the physical indicators of ripening — sugar concentration increases in the pulp, which causes differences in the osmotic pressure in the tissue. Meanwhile, the peel loses its water content due to transpiration. Hence, the pulp-to-peel ratio shows an increasing trend during the ripening process. This measurement can also be performed using the peel-to-pulp ratio, but this would show a concomitantly decreasing trend with the ripening process. In this study, bananas in the treated group exhibited a lower ratio than the control group bananas, indicating a correlation with a slower ripening process in the treated group.

3.2. Gene Expression Profile Analysis

The *MaACS1* gene expression profile analysis was conducted as described by Handayani and Dwivany (2014) using *MaGAPDH* as the reference gene (Figure 4). Results showed that the expression level of *MaACS1* in the control group was statistically significantly higher than that in the treated group ($p<0.05$). Lower *MaACS1* gene expression may result in lower ethylene synthesis, as the gene has been reported to be a member of the ACC synthase gene family that converts AdoMet into ACC with the help of the enzyme ACC synthase. As mentioned earlier, ACC is the intermediate precursor of ethylene and influenced by ethylene auto-catalytic reaction. The change of O_2 to CO_2 ratio in the atmosphere by TiO_2 treatment may result in a reduction of auto-catalytic reaction and ethylene biosynthesis. The *MaACS1* has been reported as a marker for two different treatments to prolong banana fruit ripening, such as low temperature and fungicide storage as well as chitosan coating treatment (Dwivany et al. 2016; Lustriane et al. 2018).

On the basis of previous studies, the expression level of the *MaACS1* gene increases significantly during early ripening and then decreases (Inaba et al. 2007; Karmawan et al. 2009). In this study, the expression of *MaACS1* was increased until day six and then decreased on day 8.

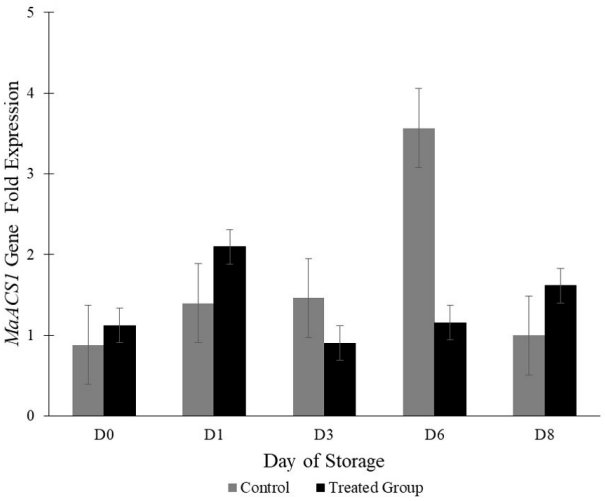

FIGURE 4 Semi quantitative *MaACS1* gene profile analysis. The observation points were choosen between the day of observation to day 8 (D-day, D-1, D-3, D-6, D-8).

4. Conclusions

This study has provided further information about the effects of using the TiO_2 chamber on the physical, physiological, and gene expression changes during banana ripening. Earlier studies have also used TiO_2 application to delay papaya and tomato ripening (Maneerat and Hayata 2006; Lourenço et al. 2017). However, to the best of our knowledge, this study is the first investigation on banana ripening using the TiO_2 chamber treatment, wherein the results provided new insights into important gene expression changes occurring during ripening and further suggested that these changes can be used as an important biomarker for evaluating banana ripening. The findings of this study can provide a novel strategy to increase the postharvest quality of banana by prolonging its shelf life. Finally, these results can also provide a better understanding of the process of banana ripening and aid the development of postharvest technology.

Acknowledgments

This research was partly funded by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia. The authors thank Banana Group-Institut Teknologi Bandung for support during this study.

Authors' contributions

FMD, RRE, and VS designed the study. ASP and AAP carried out the laboratory work, and FMD and AAP analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interest.

References

Bierhals JD, Lajolo FM, Cordenunsi BR, Oliveira Do Nascimento JR. 2004. Activity, cloning, and expression of an isoamylase-type starch-debranching enzyme from banana fruit. J Agric Food Chem. 52(24):7412–7418. doi:10.1021/jf049300g.

Cano MP, De Ancos B, Matallana MC, Cámara M, Reglero G, Tabera J. 1997. Differences among Spanish and Latin-American banana cultivars: Morphological, chemical and sensory characteristics. Food Chem. 59(3):411–419. doi:10.1016/S0308-8146(96)00285-3.

Charoenshap B, Uthaibutra J, Kumpoun W. 2012. Titanium dioxide photocatalytic oxidation on storage life extension of *Mangifera indica* cv. Nam dork mai. Agric Sci J. 43(3):331–334.

Cordeiro MCR, Silva MS, Oliviera-Filho EC, Gayoso de Miranda ZDJ, Aquino FDG, Fragoso RDR, Almeida J, Roveinia L, Andrade MD. 2008. Optimization of a Method of Total RNA Extraction from Brazilian Native Plants Rich in Polyphenols and Polysaccharides. In: Simposio Internacional Savanas Tropicais Parla Mundi, Brazil. p. 12–17. doi:10.13140/RG.2.1.3311.0162.

Dadzie BK, Orchard JE. 1997. Routine post-harvest screening of banana/plantain hybrids: criteria and methods, volume 2. Montpellier: International Plant Genetic Resources Institute.

Duan X, Joyce DC, Jiang Y. 2007. Postharvest biology and handling of banana fruit. Fresh Produce. 1(2):140–152.

Dwivany FM, Hermawaty D, Esyanti RR. 2016. 'Raja Bulu' banana *MaACS1* and *MaACO1* gene expression during postharvest storage. In: JB Golding, JA Heyes, PMA Toivonen, D Avanzato, R Harker, editors, XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): International Symposia on Postharvest Knowledge for the Future and Consumer and Sensory Driven Improvements to Fruits and Nuts. Leuven: International Society for Horticultural Science (ISHS) 1120. p. 111–114. doi:10.17660/ActaHortic.2016.1120.16.

FAO US. 2019. Top 10 Country Production of Bananas Year 2017. URL http://www.fao.org/faostat/en/{#}rankings/countries{_}by{_}commodity.

Friend J, Rhodes MJC, editors. 1981. Recent advances in the biochemistry of fruit and vegetables. London: Academic Press.

Guo H, Ecker J. 2004. The ethylene signaling pathway: new insights. Curr Opin Plant Biol. 7(1):40–49. doi:10.1016/j.pbi.2003.11.011.

Handayani RU, Dwivany F. 2014. *MaACS2*, a stress-inducible ACC synthase gene in *Musa acuminata* AAA group cultivar pisang ambon. J Math Fund Sci. 46(2):194–203. doi:10.5614/j.math.fund.sci.2014.46.2.8.

Hussain M, Bensaid S, Geobaldo F, Saracco G, Russo N. 2010. Photocatalytic degradation of ethylene emitted by fruits with TiO_2 nanoparticles. Ind Eng Chem Res. 50(5):2536–2543. doi:10.1021/ie1005756.

Inaba A, Liu X, Yokotani N, Yamane M, Lu WJ, Nakano R, Kubo Y. 2007. Differential feedback regulation of ethylene biosynthesis in pulp and peel tissues of banana fruit. J Exp Bot. 58(5):1047–1057. doi:10.1093/jxb/erl265.

Kanellis AK, Tonutti P, Perata P. 2009. Biochemical and Molecular Aspects of Modified and Controlled Atmospheres. In: EM Yahia, editor, Modified and Controlled Atmospheres for Storage, Transpiration and Packaging of Horticultural Commodities. Boca Raton: CFC Press. p. 535–567. doi:10.1201/9781420069587.

Karmawan LU, Suhandono S, Dwivany FM. 2009. Isolation of MA-ACS gene family and expression study of *MA-ACS1* gene in *Musa acuminata* cultivar pisang ambon lumut. HAYATI. 16(1):35–39. doi:10.4308/hjb.16.1.35.

Liu X, Shiomi S, Nakatsuka A, Kubo Y, Nakamura R, Inaba A. 1999. Characterization of ethylene biosynthesis associated with ripening in banana fruit. Plant Physiol. 121(4):1257–1265. doi:10.1104/pp.121.4.1257.

Lourenço RERS, Linhares AAN, de Oliveira AV, da Silva MG, de Oliveira JG, Canela MC. 2017. Photodegradation of ethylene by use of TiO_2 sol-gel on polypropylene and on glass for application in the postharvest of papaya fruit. Environ Sci Pollut Res. 24(7):6047–6054. doi:10.1007/s11356-016-8197-5.

Lustriane C, Dwivany FM, Suendo V, Reza M. 2018. Effect of chitosan and chitosan-nanoparticles on post harvest quality of banana fruits. J Plant Biotechnol 45(1):36–44. doi:10.5010/JPB.2018.45.1.036.

Maneerat C, Hayata Y. 2006. Efficiency of TiO_2 photocatalytic reaction on delay of fruit ripening and removal of off-flavors from the fruit storage atmosphere. Trans ASABE. 49(3):833–837. doi:10.13031/2013.20467.

Matile P, Hörtensteiner S, Thomas H, Kräutler B. 1996. Chlorophyll breakdown in senescent leaves. Plant Physiol. 112(4):1403. doi:10.1104/pp.112.4.1403.

Mosera S, Müller T, Holzinger A, Lütz C, Jockusch S, Turro NJ, Kräutler B. 2009. Fluorescent chlorophyll catabolites in bananas light up blue halos of cell death. Proc Natl Acad Sci USA. 106(37):15538–15543. doi:10.1073/pnas.0908060106.

Subagio A, Morita N, Sawada S. 1996. Carotenoids and their fatty-acid esters in banana peel. J Nutr Sci Vitaminol. 42(6):553–566. doi:10.3177/jnsv.42.553.

Yang SF, Hoffman NE. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann Rev Plant Physiol. 35(1):155–189. doi:10.1146/annurev.pp.35.060184.001103.

Zhu Z, Zhang Y, Shang Y, Wen Y. 2019. Electrospun nanofibers containing TiO_2 for the photocatalytic degradation of ethylene and delaying posthar-

vest ripening of bananas. Food Bioprocess Technol. 12(2):281–287. doi:10.1007/s11947-018-2207-1.