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Chitosan-based Edible Coating Prolongs *Musa troglodytarum* L. ('Pisang Tongkat Langit') Fruit Shelf-life and Changes the *ACS1* and *ACO1* Gene Expression Profile

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ABSTRACT

Musa troglodytarum L. ('Pisang Tongkat Langit'), a banana cultivar which originated from Eastern Indonesia, has an economic potential due to the high β-carotene content on its pulp. Being a climacteric fruit, *M. troglodytarum* has a short shelf-life that can reduce fruit quality. In this study, the effect of 1.25% (w/v) chitosan coating on *M. troglodytarum* fruit shelf-life and *ACS1* and *ACO1* gene expression analysis using quantitative PCR were evaluated. Results showed that the application of chitosan coating delayed the fruit ripening process for two days by delaying several fruit physical and chemical changes. *ACS1* and *ACO1* gene expression analysis showed a different expression pattern, the expression level was lower on chitosan-coated fruits on the first day compared to control. In conclusion, chitosan-based edible coating delayed *M. troglodytarum* fruit ripening and changed the *ACS1* and *ACO1* gene expression pattern, compared with the chitosan coating effect on Cavendish banana which also prolonged fruit ripening and suppressed *ACS1* and *ACO1* expression in a previous research.

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INTRODUCTION

Banana has been considered as the world's number four important food crop (Instituto de Promocion de Exportaciones e Inversiones, 2016). This fruit is widely consumed in the world due to its high nutrition. The ripe fruit has 89 kcal per 100 g fresh weight for energy source, carbohydrates, fibers, proteins, fats, calcium, iron, vitamins, and potassium (Pareek, 2015). One of the local banana cultivar from Eastern Indonesia that has an economic potential is *Musa troglodytarum* L. ('Pisang Tongkat Langit'), belongs to the *Australimusa* section (Ploetz et al., 2007). This banana pulp has a high β-carotene content (520-2,780 $\mu g \cdot 100\ g^{-1}$), a pro-vitamin A precursor (Englberger et al., 2003b). Thus, this banana could be a good source of vitamin A. Vitamin A is an essential nutrient in the human diet and it has been reported that vitamin A deficiency is a world health problem and is related to mortality rate especially in developing countries (Englberger et al., 2003a).

Banana is a climacteric fruit where ripening is associated with a sharp increase followed by a rapid decline of ethylene production in the early climacteric period (Liu et al., 1999). This condition could speed up the fruit ripening process and shorten the fruit's shelf-life. Postharvest technologies have been developed to solve this problem, such as using controlled atmosphere (CA) (Ahmad et al., 2001) and modified atmosphere packaging (MAP) (Kudachikar et al., 2011), but these technologies are relatively expensive.

An alternative postharvest technology which is relatively low cost is edible coating. Edible coating has received much attention because it is capable of forming a thin film that could prevent moisture loss and oxygen diffusion into the plant tissues, thus maintaining its postharvest quality (Jianglian & Shaoying, 2013; Xing et al., 2016). One promising edible coating biopolymer is chitosan, which is a chitin derivative that is known to be non-toxic, biodegradable, biocompatible, and biofunctional. Furthermore, chitosan is considered to be Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) (Luo & Wang, 2013).

Chitosan-based edible coating has been applied on some banana cultivars and could extend their shelf-life, such as on 'Berangan' (*Musa sapientum*, AAA group) (Malmiri et al., 2011; Maqbool et al., 2011) and Cavendish (*Musa acuminata*, AAA group) (Lustriane et al., 2018; Pratiwi et al., 2015). Though the study of chitosan effect on banana ripening had been confirmed through physical and chemical analysis, only limited study had been conducted on the molecular mechanism, such as analysis of the expression of *ACS* and *ACO* genes on chitosan-coated Cavendish banana (Dwivany et al., 2018; Lustriane et al., 2018; Yamamoto et al., 2018). *ACS* is a gene encoding 1-aminocyclopropane-1-carboxylic acid synthase (ACS), while *ACO* encodes 1-aminocyclopropane-1-carboxylic acid oxidase (ACO). These two key enzymes play an important role in ethylene biosynthesis (Xu & Zhang, 2015).

Moreover, *ACS1* and *ACO1* expression were associated with ripening process of banana fruit (Karmawan et al., 2009; Liu et al., 1999; López-Gómez et al., 1997), and these genes had been used as markers for the ripening process of chitosan-coated Cavendish banana (Dwivany et al., 2018; Lustriane et al., 2018). It was reported that these genes had been succesfully isolated and characterized from *M. troglodytarum* fruit pulp using *ACS1* and *ACO1* primers of *Musa acuminata* (AAA group) (Dwivany et al., 2020). But, there had been no report on the effect of chitosan coating to extend the shelf-life and *ACS1* and *ACO1* gene expression of *M. troglodytarum* fruit. Therefore, this study aimed to investigate the effect of chitosan coating on the shelf-life and *ACS1* and *ACO1* expression of *M. troglodytarum* fruit.

In this study, the chitosan coating concentration was 1.25% according to the optimized concentration to prolong the shelf-life of Cavendish banana at room temperature by Lustriane et al. (2018). Besides, 1% acetic acid solution, the solvent of chitosan solution, used as a coating to analyze its effect on the fruit postharvest storage, since Du et al. (1997) found that acetic acid coating affected pear fruit physical characteristics during storage.

MATERIALS AND METHODS

Materials

'Pisang Tongkat Langit' (*Musa troglodytarum* L.) mature green fruits were harvested from Ullath Village, Saparua Island, Maluku Province (Moluccas Islands), Indonesia (Figures 1a and 1b). Eight hands, which consisted of 5 until 13 fingers, were chosen randomly from eight bunches. Each bunches were derived from different herbs (Figure 1c). The fingers then were separated from each hand (Figure 1d) and selected visually for similarity in relative peel color, size, and without physical damage and infection of fungi on the fruit surface. The fingers, which weight ranged from 186.60 g to 256.32 g, were washed with commercial soap. Then the fruits were air-dried and the tips were wiped with 70% ethanol. One finger was used for each replicate, with three replicates per treatment sets per evaluation date. Meanwhile, three fingers were used for control samples. Chitosan (food graded) was purchased from Biotech Surindo, Indonesia, with high molecular weight and 85% degree of acetylation. All chemicals used in this research were analytical grade.

Preparation and Application of Chitosan Coating

Fruits were grouped into two experimental sets, i.e. 1.25% chitosan-coated and 1% acetic acid-coated fruits, and a control (uncoated fruits) set. There were three replicates per treatment sets per evaluation date. 1.25% chitosan solution was chosen according to a study conducted by Lustriane et al. (2018). Meanwhile, 1% acetic acid solution was used to examine its effect on the postharvest storage of *M. troglodytarum* fruits, since it was the main solvent for chitosan.

The 1.25% (w/v) chitosan solution and 1% (v/v) acetic acid solution were prepared

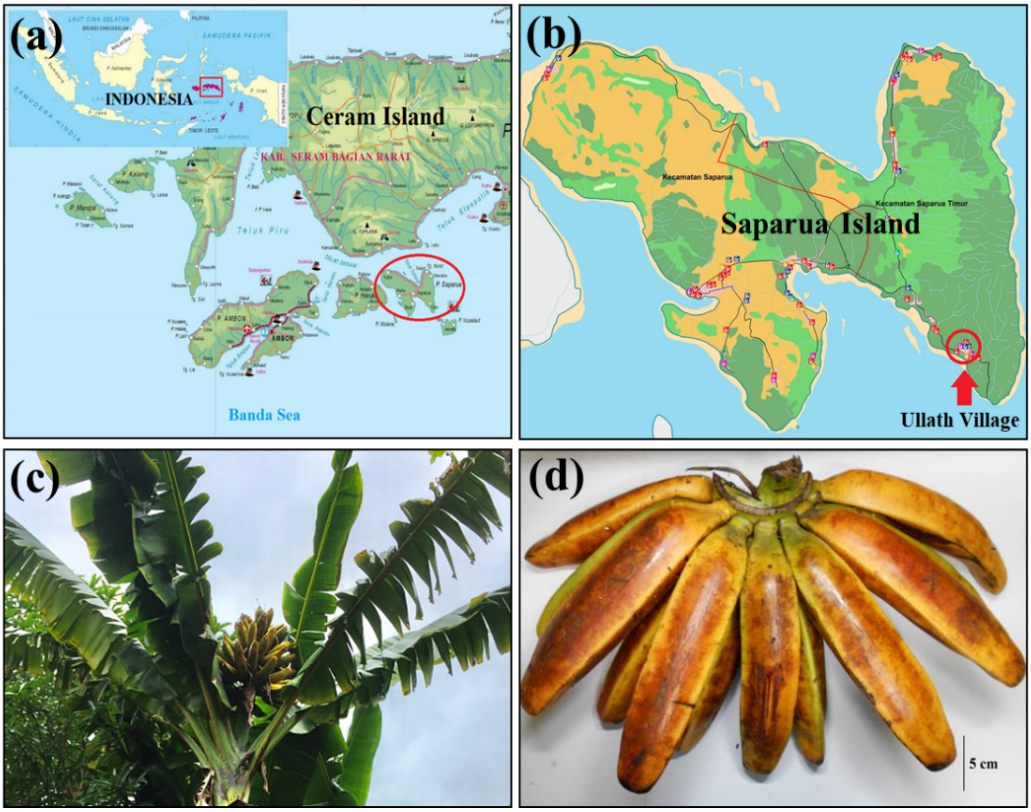


Figure 1. Sampling location of *Musa troglodytarum* L. ('Pisang Tongkat Langit'). (a) Saparua Island in Maluku Province is circled in red. The inset shows the position of the map in Indonesia [Ministry of Education and Culture of Indonesia (Kemdikbud), 2018], (b) Location of Ullath Village (red arrow) in Saparua Island (Kemdikbud, 2015), (c) *Musa troglodytarum* herb and bunches, (d) Hand of *M. troglodytarum* fruits

according to Malmiri et al. (2011) method with modification.

Briefly, 1% (v/v) acetic acid solution was prepared by dissolving glacial acetic acid in distilled water. The 1.25% (w/v) chitosan solution was prepared by dissolving the chitosan powder in 1% (v/v) acetic acid solution. Both of the solutions were agitated with a magnetic stirrer until homogenous. The pH of solution was adjusted to 5.5-5.6 by adding 3 N NaOH. Coating application was done by dipping the fruits into chitosan or acetic acid solution for 2 min and air-dried at room temperature for 2 hours. Then the fruits were stored at ambient temperature of 27±0.7°C and relative humidity of 82.57±3.62% for 9 days. The postharvest analysis was carried out on days 0, 1, 3, 5, 7, and 9 of storage.

Fruit Physical Analysis

Observation of the Peel and Pulp of the Fruit. Changes on the peel and pulp of the fruit were noted and photographed at each

observation days of storage, which was described by Dadzie and Orchard (1997).

Weight Loss Determination. Weight loss was measured, which was described by Dadzie and Orchard (1997) as well as Lustriane et al. (2018), by comparing the initial fruit weight (day 0) and the fruit weight on each observation days during storage. The weight loss percentage was obtained by calculating the difference between the initial weight and final weight, then dividing the result with the initial weight and multiplying with 100%.

Pulp to Peel Ratio. The middle part of the fruit was cut transversely 1.0 cm thick, then the peel and pulp were weighed separately. The pulp to peel ratio was calculated by dividing the pulp weight with the peel weight (Dadzie & Orchard, 1997).

Fruit Chemical Analysis

Starch into Sugar Coversion. Starch into sugar conversion analysis was conducted using iodine starch staining techniques developed by Dadzie and Orchard (1997). The iodine staining solution was made by dissolving 1% potassium iodide and 0.25% iodine in warm distilled water. The middle part of the fruit was cut transversely 1.0 cm thick, then the peel was separated from the pulp. Then the pulp was immersed at a depth of 5 mm for 20-30 seconds in the staining solution. The iodine starch pattern of each fruit was compared with the chart of banana pulp stained surface with iodine staining (Blankenship et al., 1993).

Total Soluble Solids (TSS). Total soluble solids (TSS) content of fruit pulp was measured by using a refractometer (ATAGO) which was described by Dadzie and Orchard (1997). Briefly, 15 g fruit pulp was homogenized with 45 mL of distilled water using a blender to give three times dilution. The homogenate was centrifuged at (18,600 x *g*) for 5 min using microcentrifuge (Thermo Fisher Scientific™ Heraeus Pico™ 21). Then a few drops of the supernatant were dripped on the refractometer prism before reading. The results were multiplied by three and expressed as degree Brix (^{o}Brix). Calibration of the refractometer was done before measurement by placing a few drops of distilled water to give a 0 ^{o}Brix reading.

Fruit Surface Microstructure Analysis

The 1.25% chitosan-coated and uncoated fruits on day 1 after storage (unripe fruit) were used for fruit surface microstructure analysis, as described by Lustriane et al. (2018) with slight modification. Square pieces (0.5 cm x 0.5 cm) of 2 mm thick peel from the fruits were cut transversely from the middle part of the fruit. The samples were freeze dried (freeze dryer VD-550R, Taitec Corporation) for 25 hours, then were coated with gold (Au) using ion sputter coater (MC1000, Hitachi). The microstructure of peel outer surface was analyzed by using scanning electron microscopy (JSM-6510A, JEOL Ltd.) using an accelerating voltage of 10 kV and viewed in 1,000x zoom.

Sensory Quality Evaluation

Sensory quality evaluation was conducted using hedonic test which was described by Amerine et al. (1965). Sixteen semi-trained panelists were chosen to evaluate untreated (control) and treated fruits (days 5, 6, and 7 of storage at 27±0.7°C). Fruit samples were presented randomly to the panelists and rated on a seven-point hedonic scale (1 = extremely dislike, 7 = extremely like) for aroma, taste, and fruit overall acceptability.

Isolation of Total RNA and cDNA Synthesis

RNA isolation was carried out on uncoated (control) and 1.25% chitosan-coated fruits on observation days of 0, 1, 3, 5, and 7. Total RNA was isolated from fruit pulp using Cordeiro's method (Corderio et al., 2008). cDNA synthesis was done by using the isolated RNA as template and iScript™ cDNA Synthesis (Bio-Rad Laboratories) as mix reagent.

***ACS1* and *ACO1* Gene Expression Analysis**

Total cDNA from the pulp of uncoated and 1.25% chitosan-coated fruits were used for *ACS1* and *ACO1* gene expression analysis by quantitative PCR (qPCR). Specific primers were used to amplify the cDNA fragments of *MaACS1* (primer of Cavendish banana *ACS1* gene), *MaACO1* (primer of Cavendish banana *ACO1* gene), and a reference gene, *MaGAPDH* (primer of Cavendish banana *GAPDH* gene). The primer pairs used to amplify *ACS1*, *ACO1*, and *GAPDH* are presented in Table 1. The qPCR was performed by MyGo Pro® real time PCR instrument connected with MyGo Pro PCR software, using Thunderbird® SYBR® qPCR mix reagent (Toyobo). In each qPCR analysis, three samples were used for

Table 1

Primer sequences of analyzed genes

Gene Name	Primer Name	Primer Sequences	Reference
MaGAPDH (Reference Gene)	*MaGAPDH* Forward	5'-TCAACGACCCCTTCATCAC-3'	Karmawan et al. (2009)
	MaGAPDH Reverse	5'-AGCAGCCTTGTCCTTGTCA-3'	
MaACS1	*MaACS1* Forward	5'-CCGAGACTGGATGAAGAAGAA-3'	Karmawan et al. (2009)
	MaACS1 Reverse	5'-GTCTGGGTCAAATCTGGCTC-3'	
MaACO1	*MaACO1* Forward	5'-CGAGATGCTTGCGAGAAATGG-3'	Dwivany et al. (2018)
	MaACO1 Reverse	5'-TGCAGCAAATTCCTTCATCGC-3'	

triplicate. The qPCR cycle condition was set at 95°C initial hold for 60 s, followed by 40 cycles of denaturation (95°C, 15 s), annealing (60°C, 30 s), and extension (72°C, 30 s). This was followed by a melting stage from 60°C to 97°C (4°C increments for 30 s each at the initial stage and 0.5°C increments for 1 s each at the final stage).

Gene expression levels were normalized using the reference gene. Then the relative gene expression levels were calculated using obtained Ct (cycle threshold) value by $2^{-\Delta\Delta Ct}$ method as formulated by Livak and Schmittgen (2001).

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics ver. 20. Analysis of variance (ANOVA) was used to measure the treatment effect and followed by Tukey's HSD as *post hoc* test. Differences were considered to be significant when the *P*-values ≤ 0.05.

RESULTS AND DISCUSSION

Fruit Physical Analysis

Changes on Peel and Pulp Color. Changes on peel and pulp color are often used to estimate fruit ripening stages. The effects of coatings on peel and pulp color changes are presented in Figure 2a. As observed, peel color of uncoated (control) and 1% acetic acid-coated fruit were yellowish orange on day 3 and orange on day 7, while 1.25% chitosan-coated peel color just changed to yellowish orange on day 5 and orange on day 9. Moreover, control and 1% acetic acid-coated fruit showed a faster deterioration compared with 1.25% chitosan-coated fruit on day 9. Observation of the pulp color showed that the pulp color of the control fruit changed from yellow to orange on day 3, this color was obtained on day 5 for the 1% acetic acid-coated fruit. On 1.25% chitosan-coated fruit, the pulp reached the orange color on day 9. These results indicated that chitosan coating delayed the changes in the peel and pulp color.

Chitosan coating formed a thin film on fruit surface which can be seen on microstructure observation (Figure 4). This thin film provides a physical barrier against water and gas, which causes a reduction in the diffusion of O_2 into and CO_2 out from the plant tissue (Xing et al., 2016). If internal O_2 decreases, ethylene biosynthesis is inhibited and ripening process is slowed down, including chlorophyll degradation on peel and pulp (Knee, 1980). Chitosan coating may also inhibit carotenoid pigment production in the fruit. This may happen since the carotenoid production is affected by ethylene (Rodrigo & Zacarias, 2007). Delay on peel color changes are also observed on other chitosan-coated banana fruits, such as 'Berangan' (Maqbool et al., 2011) and Cavendish (Lustriane et al., 2018). Chitosan coating also delays fruit decay because of its antimicrobial properties (Jianglian & Shaoying, 2013; Xing et al., 2016). Delay on fruit decay was also observed on chitosan-coated raspberry (Tezotto-Uliana et al., 2014) and Cavendish banana (Lustriane et al., 2018). On Cavendish banana, it was reported that chitosan coating (concentration

1.25%) delayed the fruit deterioration until 2-3 days (Lustriane et al., 2018).

Weight Loss. The effect of coatings on weight loss is shown in Figure 2b. Fruit weight loss is one of important parameter to determine fruit postharvest quality (Maqbool et al., 2011). Generally weight loss increases during ripening. However, according to statistical analysis, all of the treatments did not show any significant differences ($P \leq 0.05$). This result showed that chitosan coating did not affect weight loss of *M. troglodytarum* fruit. Fruit weight loss is related with moisture loss from respiration and transpiration during ripening. Chitosan coating is supposed to prevent moisture loss and weight loss of fruits (Eshghi et al., 2014). Therefore, chitosan coating can be added with a plasticizer such as glycerol to increase its permeability to water so it can restrain transpiration and decrease fruit weight loss (Malmiri et al., 2011).

Pulp to Peel Ratio. The effect of coatings on pulp to peel ratio can be seen on Figure 2c. Generally, pulp to peel ratio increased until it reached a peak, then it decreased at the end of the ripening process. The peak values of each treatment were significantly different ($P \leq 0.05$) from one another. Each treatment reached its peak value at different storage time, 1% acetic acid-coated fruit on day 5, control on day 7, and 1.25% chitosan-coated fruit on day 9. This suggested that chitosan coating slowed down the changes in the pulp to peel ratio.

During fruit ripening, pulp and peel tissues undergo changes in water and soluble sugar content, which lead to osmotic pressure differences between pulp and peel. Therefore, the pulp and peel weight ratio is increased (Dadzie & Orchard, 1997). Chitosan coating slows down starch conversion into soluble sugar (Maqbool et al., 2011). Hence, osmosis from pulp to peel is inhibited and pulp to peel ratio changes is delayed. Lustriane et al. (2018) and Pratiwi et al. (2015) reported that pulp to peel ratio of chitosan-coated Cavendish banana was lower than the uncoated banana.

Fruit Chemical Analysis

Starch into Sugar Conversion Analysis. The effects of coatings on changes of starch degradation pattern are visualized in Figure 3a. Starch degradation in 1.25% chitosan-coated fruit pulp was the slowest among all treatments. This could be seen from the presence of starch staining on the middle part (placenta) and the edge part of the 1.25% chitosan-coated fruit pulp on day 5, while control and 1% acetic acid-coated fruit only showed starch staining on the edge of the pulp.

During fruit ripening, starch is degraded into soluble sugar by amylase activity, hence this decreases the starch content and increases the soluble sugar content of the pulp (do Nascimento et al., 2006). In chitosan-coated fruit, ethylene production is inhibited (Maqbool et al., 2011). As a result, starch degradation rate decreases, since amylase activity is affected by

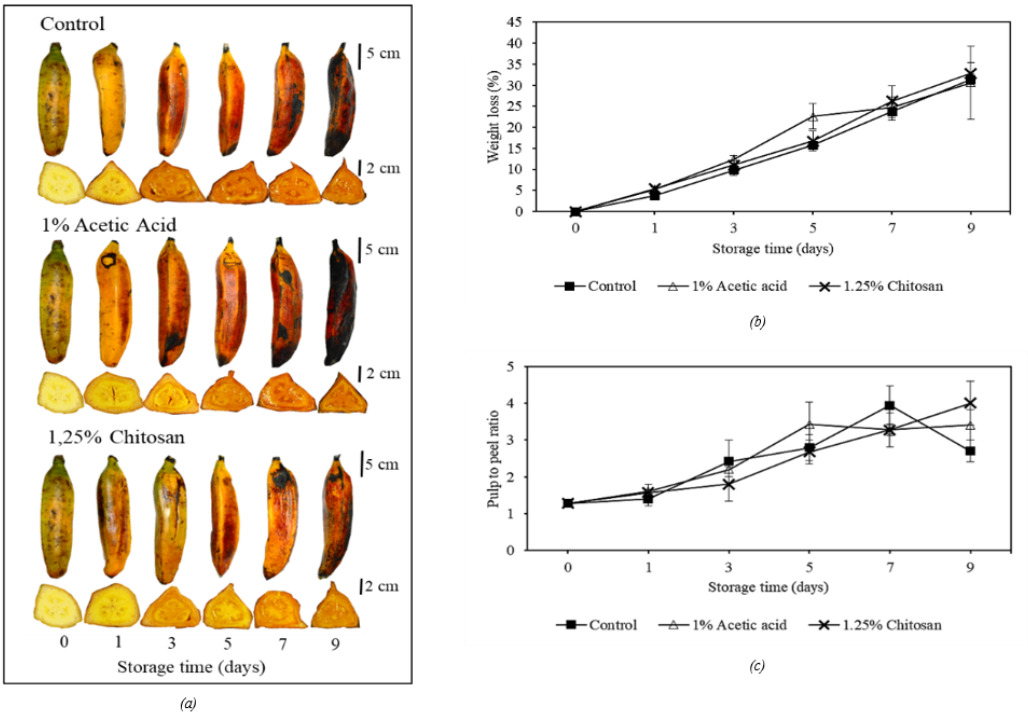
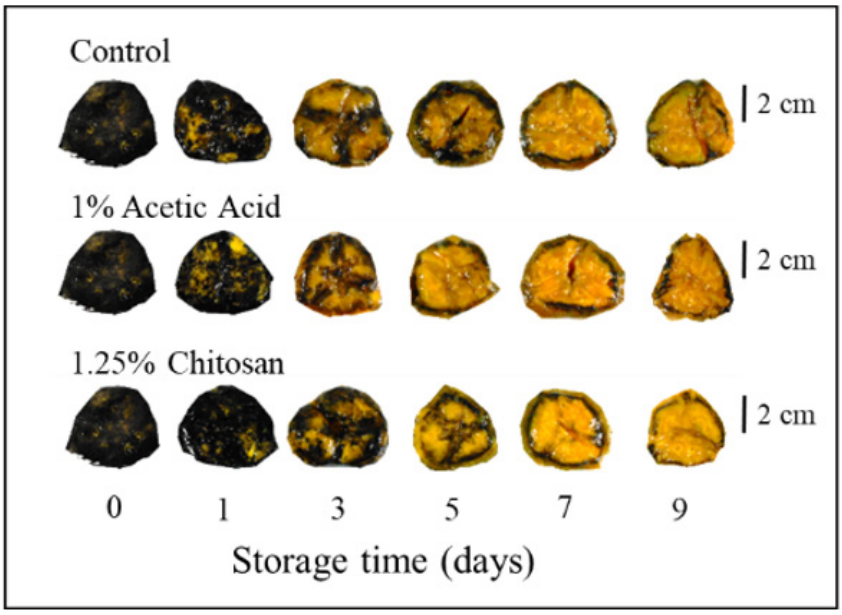


Figure 2. Changes of the physical characteristics of uncoated (control), 1% acetic acid-coated, and 1.25% chitosan-coated *Musa troglodytarum* fruits during ripening. (a) Changes on the peel and pulp colors, (b) Weight loss (significant at $P \leq 0.05$), (c) Pulp to peel ratio (significant at $P \leq 0.05$). Error bars indicate standard deviation (SD) (n = 3)

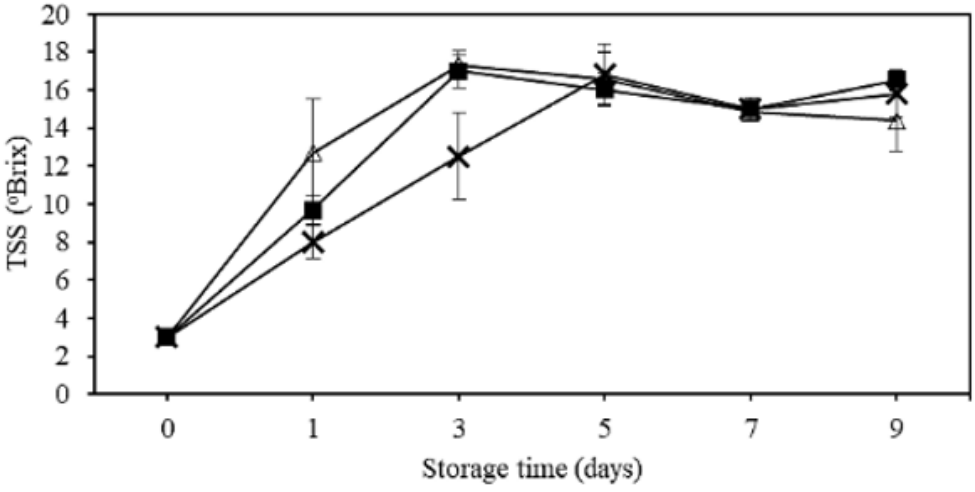
ethylene (do Nascimento et al., 2006). The effect of chitosan coating on delay of starch degradation were also reported on 'Berangan' (Maqbool et al., 2011) and Cavendish bananas (Lustriane et al., 2018).

Total Soluble Solids (TSS). The effects of coatings on total soluble solids (TSS) content during ripening are shown in Figure 3b. Generally, all treatments showed an increase of TSS until day 3 or day 5, and then followed by constant values until day 9. TSS on 1.25% chitosan-coated fruit was lower ($P \leq 0.05$) than control and 1% acetic acid-coated fruit from day 0 until day 3. However, TSS was not significantly different ($P \leq 0.05$) between all treatments on day 5 until day 9. This result showed that chitosan coating decreased TSS values of *M. troglodytarum* fruit until day 3.

Total Soluble Solids, which consists of soluble sugar and organic acids, increases in fruit pulp during ripening (Dadzie & Orchard, 1997). It is caused by starch degradation into soluble sugar (do Nascimento et al., 2006) and production of organic acids (McGlasson & Wills, 1972). In chitosan-coated fruit, there is a decrease in



(a)



(b)

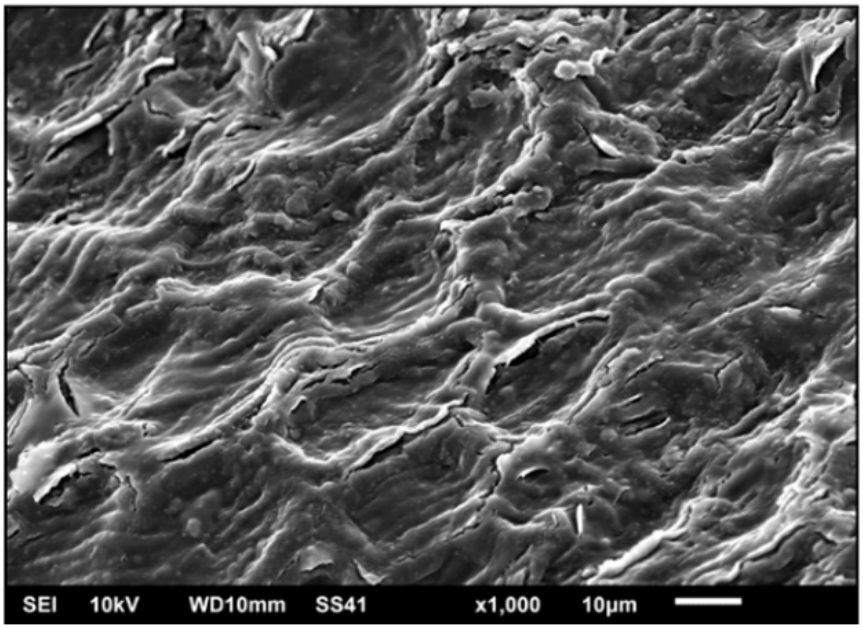
Figure 3. Changes on the chemical characteristics of uncoated (control), 1% acetic acid-coated, and 1.25% chitosan-coated *Musa troglodytarum* fruits during ripening. (a) Changes of the starch pattern on the pulp, (b) Total soluble solids of the pulp (significant at $P \leq 0.05$). Error bars indicate standard deviation (SD) (n = 3)

internal O_2 (Ali et al., 2011). Low O_2 content decreases soluble sugar concentrations (Ali et al., 2011) and Krebs cycle related organic acids content (citrate, malate, glutamate, and aspartate acid) (McGlasson & Wills, 1972). The effect of chitosan coating on the decrease of TSS had also been reported on papaya (Ali et al., 2011) and 'Berangan' banana (Malmiri et al., 2011).

According to physical and chemical analysis, chitosan-coated fruit shows delay on its ripening compared with control and acetic acid-coated fruits which show no delay. Meanwhile, acetic acid-coated fruit shows no significant differences with control, except for the pulp to peel ratio, which may be caused by the damaging effect on the peel by acetic acid as described by Du et al. (1997). Therefore, chitosan coating and control coating are used for fruit surface microstructure and quantitative PCR analysis.

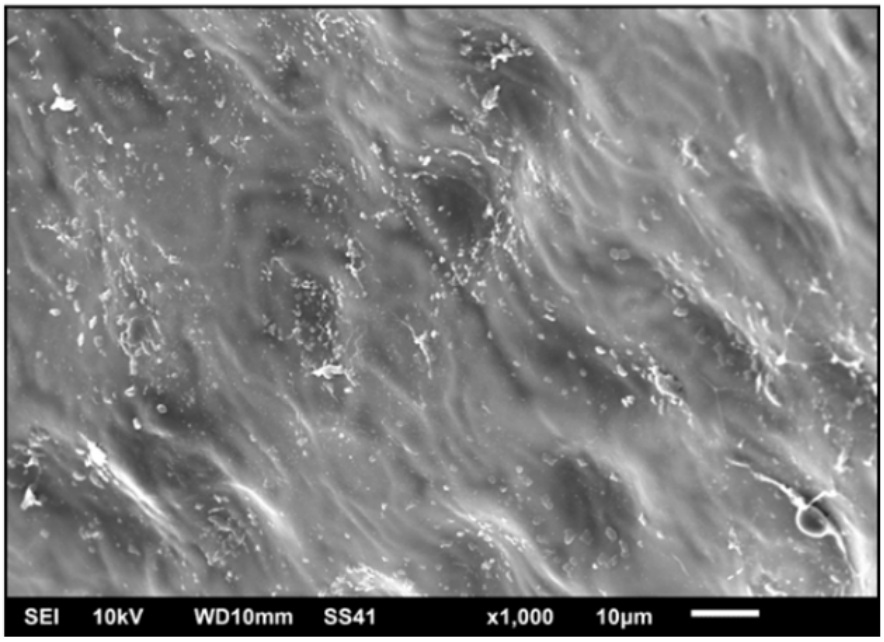
Fruit Surface Microstructure Analysis

Surface microstructure of uncoated (control) and 1.25% chitosan-coated *M. troglodytarum* fruits at day 1 of storage are shown in Figure 4. We used this stage to examine the thin film formation on the chitosan-coated fruit peel surface after one day of the coating process and compared it with the uncoated fruit (control). The microstructure of uncoated fruit showed porous surface and epidermal cells clearly. Meanwhile, chitosan-coated fruit surface was coated with a film; hence the pores and the epidermal cells were covered. Chitosan



(a)

Figure 4. SEM micrographs of *Musa troglodytarum* peel surface at day 1 of fruit storage (1,000x zoom) (a) Uncoated (control) fruit peel surface,



(b)

Figure 4. SEM micrographs of *Musa troglodytarum* peel surface at day 1 of fruit storage (1,000x zoom). (b) The 1.25% chitosan-coated fruit peel surface, which shows a thin film formation

coating formed a thin film which provided a barrier against gas and water, thus modifying internal O_2 and CO_2 in the fruit tissues (Xing et al., 2016). This condition decreased respiration and ethylene biosynthesis rate, hence decreasing the decay rate, peel color change, pulp to peel ratio, TSS, and delayed starch degradation of chitosan-coated *M. troglodytarum* fruits, which were also observed on chitosan-coated Cavendish bananas (Lustriane et al., 2018).

Sensory Quality Evaluation

The effects of coatings on sensory quality are shown in Table 2. According to panelists, the chitosan-coated fruit had lower ($P \leq 0.05$) aroma and taste quality compared with control on day 7. Meanwhile, uncoated and coated fruits showed no significant differences ($P \leq 0.05$) on overall acceptability on days 5, 6, and 7. These results indicated that chitosan coating could only keep the overall fruit quality, but not the aroma and taste quality on the last day of storage. This might have happened since the chitosan film was too thick and resulted in CO_2 accumulation in the fruit tissues, hence inducing anaerobic respiration and ethanol production which might have affected the fruit aroma and taste on the last day of storage (Xing et al., 2016).

Table 2

Sensory quality evaluation of coated and uncoated Musa troglodytarum *L. fruits at days 5, 6, and 7 of storage at 27±0.7°C*

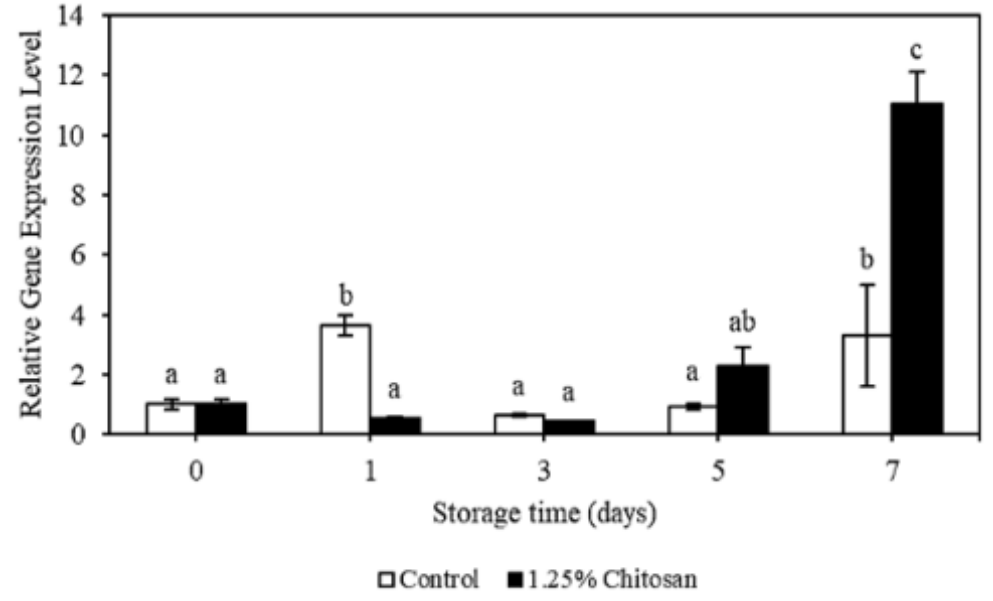
Parameter	Storage Time (Days)	Treatment		
		Control	1% Acetic Acid	1.25% Chitosan
Aroma	5	4.48 ± 0.60 a	4.50 ± 0.53 a	4.23 ± 0.75 a
	6	3.98 ± 1.07 a	4.06 ± 1.22 ab	4.15 ± 1.17 a
	7	4.13 ± 0.87 a	3.88 ± 0.65 ab	3.29 ± 0.76 b
Taste	5	4.42 ± 1.00 a	4.35 ± 0.83 a	4.44 ± 0.76 a
	6	4.46 ± 1.03 a	4.81 ± 0.92 a	4.50 ± 1.33 a
	7	4.40 ± 0.96 a	4.02 ± 1.04 ab	3.44 ± 1.11 b
Overall Acceptability	5	3.71 ± 1.77 a	3.44 ± 1.41 a	3.77 ± 1.11 a
	6	3.50 ± 1.45 a	3.00 ± 1.53 a	3.81 ± 1.25 a
	7	3.58 ± 1.15 a	3.29 ± 0.86 a	3.69 ± 1.26 a

Note. Scales 1 to 7 (1 = extremely dislike, 7 = extremely like). Mean values ± standard deviation in the same column with a same letter are not significantly different ($P \leq 0.05$) (n = 3)

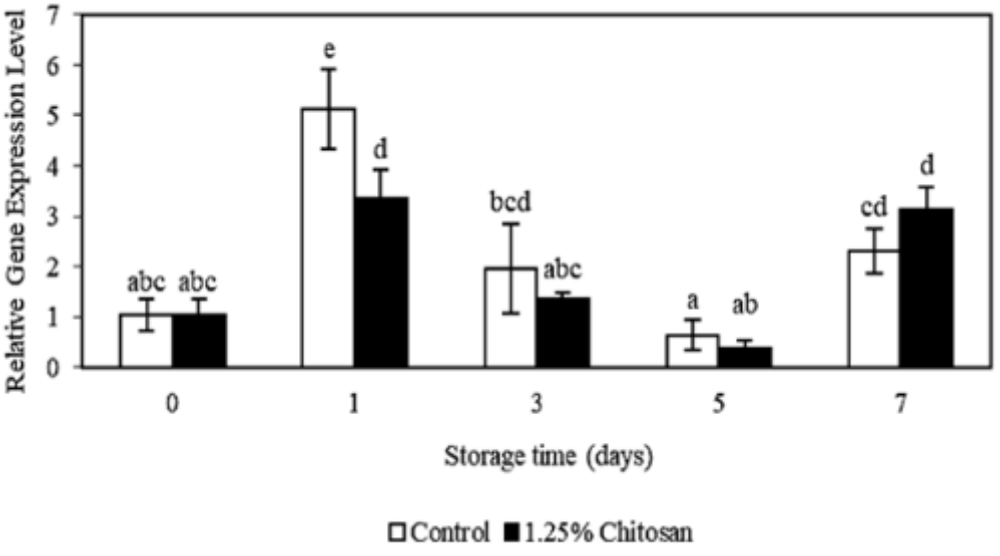
Expression Analysis of *ACS1* and *ACO1*

The effect of chitosan coating on *ACS1* and *ACO1* expression of *M. troglodytarum* fruits are presented in Figure 5. In this analysis, only uncoated (control) and chitosan-coated samples were used, since the physical and chemical analysis result of control and acetic acid-coated samples showed no significant differences. On uncoated banana (control), *ACS1* and *ACO1* expression level increased until day 1, decreased on day 3 and day 5, then increased again on day 7 although it was smaller than day 1. This expression pattern is also found on the other banana as biphasic peaks and considered as a unique pattern among banana (Lustriane et al., 2018; Pathak et al., 2003). Furthermore, the biphasic expression level pattern on *ACS1* is concomitant with biphasic ethylene production and respiration rate (Pathak et al., 2003). However, Pathak et al. (2003) explained that the mechanism on this biphasic pattern was still not understood and needed to be explored.

ACS1 and *ACO1* expression level was lower ($P \leq 0.05$) on chitosan-coated fruit compared with control on day 1. However, there were no significant differences ($P \leq 0.05$) between control and chitosan-coated fruit on day 3 and day 5. On day 7, *ACS1* expression level on chitosan-coated fruit was higher ($P \leq 0.05$) than control, while *ACO1* expression level showed no significant differences ($P \leq 0.05$) compared with control. This suggested that 1.25% chitosan coating suppressed the expression



(a)



(b)

Figure 5. Chitosan coating effect on (a) *ACS1* and (b) *ACO1* expression level of *Musa troglodytarum* fruit pulp during storage at 27±0.7°C for 7 days. Bars marked with a same letter are not significantly different ($P \leq 0.05$). Error bars indicate standard deviation (SD) (n = 3)

of *ACS1* and *ACO1* in the early ripening stage (day 1) and increased *ACS1* expression in the late ripening stage (day 7).

Chitosan coating caused the decline of ethylene production rate on banana (Maqbool et al., 2011). This condition may affect the suppression of *ACS1* and *ACO1* expression level on the early ripening stage since ethylene influenced *ACS* and *ACO* expression (Liu et al., 1999; López-Gómez et al., 1997). The same expression pattern was also observed in Cavendish banana (Lustriane et al., 2018). Low *ACS1* and *ACO1* expression level from day 1 to day 3 might decrease ethylene production, hence slowing the changes in peel and pulp color, pulp to peel ratio, starch content, and TSS on chitosan-coated fruits in the early ripening stage (day 1 until day 3).

The rise of *ACS1* and *ACO1* expression level on day 7 (Figures 5a and 5b) might be caused by the physical barrier effect of chitosan film against ethylene diffusion (Xing et al., 2016). Consequently, internal ethylene might be trapped and accumulated inside chitosan-coated fruits at the late ripening stage. Ethylene was autocatalytic at climacteric period, which means the additions of ethylene would trigger ethylene biosynthesis (McMurchie et al., 1972). Therefore, ethylene accumulation on the last ripening stage (day 7) may trigger *ACS1* and *ACO1* expression and more ethylene production, causing the peel and pulp color, pulp to peel ratio, starch content, and TSS on chitosan-coated fruit to show no differences compared to control on the last day of storage. The increase of *ACS1* expression level at the late ripening stage was also observed on chitosan-coated Cavendish banana (Dwivany et al., 2018).

On day 7, *ACS1* expression level on chitosan-coated fruits increased dramatically compared with control. However, *ACO1* expression level also raised but showed no significant differences compared with control. This might be due to low O_2 level caused by the physical barrier from chitosan coating. *ACO* is a gene encoding ACO enzyme that converts ACC to ethylene with the presence of O_2 (Xu & Zhang, 2015). Therefore, low O_2 might cause the ACO activity and *ACO1* expression level to be lowered and showed no sharp increase compared with *ACS1* expression level. Moreover, *ACS1* showed higher mRNA accumulation in the ripening process and ethylene presence than *ACO1* as reported by Liu et al. (1999). Measurement of ethylene production should be conducted to clear this mechanism.

CONCLUSION

Chitosan coating could be an alternative postharvest technology with a potential to prolong *M. troglodytarum* ('Pisang Tongkat Langit') shelf-life, because it maintained several postharvest qualities, i.e. peel and pulp condition, pulp to peel ratio, starch content, and TSS in the early ripening stage. Chitosan coating also affected fruit ripening at the molecular level, i.e. suppressed *ACS1* and *ACO1* gene expression in the early ripening stage. As compared with Cavendish banana, chitosan coating (concentration

1.25%) also prolonged the banana shelf-life until the eleventh day of storage and suppressed *ACS1* and *ACO1* gene expression (Lustriane et al., 2018).

Further gene expression analysis related with *M. troglodytarum* ethylene biosynthesis, signaling, and perception, and fruit ripening is required to reveal the mechanism clearly. In the future, chitosan coating can be developed and modified with the addition of plasticizer and natural antimicrobial compounds to get more optimal result in delaying *M. troglodytarum* fruit ripening.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Ahmad, S., Thompson, A. K., Asi, A. A., Khan, M., Chatha, G. A., & Shahid, M. A. (2001). Effect of reduced O_2 and increased CO_2 (controlled atmosphere storage) on the ripening and quality of ethylene treated banana fruit. *International Journal of Agriculture and Biology*, *3*(4), 486-490.

Ali, A., Muhammad, M. T. M., Sijam, K., & Siddiqui, Y. (2011). Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (*Carica papaya* L.) fruit during cold storage. *Food Chemistry*, *124*(2), 620-626. doi: 10.1016/j.foodchem.2010.06.085

Amerine, M. A., Pangborn, R. M., & Roessler, E. B. (1965). *Principles of sensory evaluation of food*. New York, NY: Academic Press. doi: 10.1016/C2013-0-08103-0

Blankenship, S. M., Ellsworth, D. D., & Powell, R. L. (1993). A ripening index for banana fruit based on starch content. *HortTechnology*, *3*(3), 338-339. doi: 10.21273/horttech.3.3.338

Corderio, M. C. R., Silva, M. S., Oliveira-Filho, E. C., de Miranda, Z. J. G., Aquino, F. G., Fragoso, R. R., … Andrade, L. R. M. (2008). *Optimization of a method of total RNA extraction from Brazilian native plants rich in polyphenols and polysaccharides*. Retrieved July 22, 2020, from https://www.google.comurl?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwjr9PufkMXsAhVPcCsKHcOcDmgQFjACegQIAhAC&url=http%3A%2F%2Fsimposio.cpac.embrapa.br%2Fsimposio%2Ftrabalhos_pdf%2F00539_trab2_ap.pdf&usg=AOvVaw1MKeG2TPOBxvLuk_L91hO6

Dadzie, B. K., & Orchard, J. E. (1997). *Routine post-harvest screening of banana/plantain hybrids: Criteria and methods*. Rome, Italy: International Network for Banana and Plantain (INIBAP).

Du, J., Gemma, H., & Iwahori, S. (1997). Effects of chitosan coating on the storage of peach, Japanese pear, and kiwifruit. *Journal of the Japanese Society for Horticultural Science*, *66*(1), 15-22. doi: 10.2503/jjshs.66.15

Dwivany, F. M., Aulina, A., & Pratiwi, A. S. (2018). Effects of fruit storage chamber (FSC) and chitosan coating on cavendish banana (*Musa acuminata* AAA Group) *MaACO* and *MaACS1* genes expression. *IOP Conference Series: Earth and Environmental Science*, *197*(1), 012002. doi: 10.1088/1755-1315/197/1/012002

Dwivany, F. M., Patty, K. L., & Novianti, C. (2020). Isolation and characterization of 'Pisang Tongkat Langit' (*Musa troglodytarum* L.) *ACS1* and *ACO1* gene expression during fruit ripening process. *Malaysian Applied Biology*, *49*(2), 81-92.

Englberger, L., Darnton-Hill, I., Coyne, T., Fitzgerald, M. H., & Marks, G. C. (2003a). Carotenoid-rich bananas: A potential food source for alleviating vitamin A deficiency. *Food and Nutrition Bulletin*, *24*(4), 303-318. doi: 10.1177/156482650302400401

Englberger, L., Schierle, J., Marks, G. C., & Fitzgerald, M. H. (2003b). Micronesian banana, taro, and other foods: Newly recognized sources of provitamin A and other carotenoids. *Journal of Food Composition and Analysis*, *16*(1), 3-19. doi: 10.1016/S0889-1575(02)00133-3

Eshghi, S., Hashemi, M., Mohammadi, A., Badii, F., Mohammadhoseini, Z., & Ahmadi, K. (2014). Effect of nanochitosan-based coating with and without copper loaded on physicochemical and bioactive components of fresh strawberry fruit (*Fragaria* x *ananassa* Duchesne) during storage. *Food and Bioprocess Technology*, *7*(8), 2397-2409. doi: 10.1007/s11947-014-1281-2

Instituto de Promocion de Exportaciones e Inversiones. (2016). *Sector analysis: Banano 2016*. Portoviejo, Ecuador: Pro Ecuador.

Jianglian, D., & Shaoying, Z. (2013). Application of chitosan based coating in fruit and vegetable preservation: A review. *Journal of Food Processing and Technology*, *4*(5), 5-8. doi: 10.4172/2157-7110.1000227

Karmawan, L. U., Suhandono, S., & Dwivany, F. M. (2009). Isolation of *MA-ACS* gene family and expression study of *MA-ACS1* gene in *Musa acuminata* cultivar 'Pisang Ambon Lumut'. *HAYATI Journal of Biosciences*, *16*(1), 35-39. doi: 10.4308/hjb.16.1.35

Knee, M. (1980). Physiological responses of apple fruits to oxygen concentrations. *Annals of Applied Biology*, *96*(2), 243-253. doi: 10.1111/j.1744-7348.1980.tb02985.x

Kudachikar, V. B., Kulkarni, S. G., & Prakash, M. N. K. (2011). Effect of modified atmosphere packaging on quality and shelf life of 'Robusta' banana (*Musa* sp.) stored at low temperature. *Journal of Food Science and Technology*, *48*(3), 319-324. doi: 10.1007/s13197-011-0238-y

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

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, *25*(4), 402-408. doi: 10.1006/meth.2001.1262

López-Gómez, R., Campbell, A., Dong, J. G., Yang, S. F., & Gómez-Lim, M. A. (1997). Ethylene biosynthesis in banana fruit: Isolation of a genomic clone to ACC oxidase and expression studies. *Plant Science*, *123*(1-2), 123-131. doi: 10.1016/S0168-9452(97)04578-0

Luo, Y., & Wang, Q. (2013). Recent advances of chitosan and its derivatives for novel applications in food science beverages. *Journal of Food Processing and Beverages*, *1*(1), 1-13. doi: 10.13188/2332-4104.1000002

Lustriane, C., Dwivany, F. M., Suendo, V., & Reza, M. (2018). Effect of chitosan and chitosan-nanoparticles on post harvest quality of banana fruits. *Journal of Plant Biotechnology*, *45*(1), 36-44. doi: 10.5010/JPB.2018.45.1.036

Malmiri, H. J., Osman, A., Tan, C. P., & Abdul Rahman, R. (2011). Development of an edible coating based on chitosan-glycerol to delay 'Berangan' banana (*Musa sapientum* cv. Berangan) ripening process. *International Food Research Journal*, *18*(3), 989-997.

Maqbool, M., Ali, A., Alderson, P. G., Zahid, N., & Siddiqui, Y. (2011). Effect of a novel edible composite coating based on gum arabic and chitosan on biochemical and physiological responses of banana fruits during cold storage. *Journal of Agricultural and Food Chemistry*, *59*(10), 5474-5482. doi: 10.1021/jf200623m

McGlasson, W. B., & Wills, R. B. H. (1972). Effects of oxygen and carbon dioxide on respiration, storage life, and organic acids of green bananas. *Australian Journal of Biological Sciences*, *25*(1), 35-42. doi: 10.1071/BI9720035

McMurchie, E. J., McGlasson, W. B., & Eaks, I. L. (1972). Treatment of fruit with propylene gives information about the biogenesis of ethylene. *Nature*, *237*(5352), 235-236. doi: 10.1038/237235a0

Ministry of Education and Culture of Indonesia [Kemdikbud]. (2015). *Peta sebaran satuan pendidikan Kecamatan Saparua, Kabupaten Maluku Tengah* [Map of the distribution of education units in Saparua District, Central Maluku Regency]. Retrieved February 17, 2019, from http://spasial.data.kemdikbud.go.id/index.php/cpetatematik/display/0F44734E-0027-4FE7-9E41-B29D6E6276B7

Ministry of Education and Culture of Indonesia. [Kemdikbud]. (2018). *Sasi Maluku - Peta budaya rumah belajar* [Sasi Maluku - Map of learning house culture]. Retrieved September 28, 2018, from https://petabudaya.belajar.kemdikbud.go.id/Repositorys/SasiMaluku/

do Nascimento, J. R. O., Júnior, A. V., Bassinello, P. Z., Cordenunsi, B. R., Mainardi, J. A., Purgatto, E., & Lajolo, F. M. (2006). Beta-amylase expression and starch degradation during banana ripening. *Postharvest Biology and Technology*, *40*(1), 41-47. doi: 10.1016/j.postharvbio.2005.11.008

Pareek, S. (2015). Nutritional and biochemical composition of banana (*Musa* spp.) cultivars. In M. Simmonds & V. Preedy (Eds.), *Nutritional composition of fruit cultivars* (pp. 49-81). New York, NY: Academic Press. doi: 10.1016/B978-0-12-408117-8.00003-9

Pathak, N., Asif, M. H., Dhawan, P., Srivastava, M. K., & Nath, P. (2003). Expression and activities of ethylene biosynthesis enzymes during ripening of banana fruits and effect of 1-MCP treatment. *Plant Growth Regulation*, *40*(1), 11-19. doi: 10.1023/A:1023040812205

Ploetz, R. C., Kepler, A. K., Daniells, J., & Nelson, S. C. (2007). Banana and plantain — An overview with emphasis on Pacific island cultivars. Part 1 : Taxa in the Musaceae. In C. R. Elevitch (Ed.), *Species profiles for Pacific island agroforestry* (pp. 1-27). Hōlualoa, Hawaii: Permanent Agriculture Resources (PAR).

Pratiwi, A. S., Dwivany, F. M., Larasati, D., Islamia, H. C., & Martien, R. (2015). *Effect of chitosan coating and bamboo FSC (fruit storage chamber) to expand banana shelf life*. Retrieved July 22, 2020, from https://aip.scitation.org/doi/abs/10.1063/1.4930763?journalCode=apc

Rodrigo, M. J., & Zacarias, L. (2007). Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (*Citrus sinensis* L. Osbeck) fruit. *Postharvest Biology and Technology*, *43*(1), 14-22. doi: 10.1016/j.postharvbio.2006.07.008

Tezotto-Uliana, J. V., Fargoni, G. P., Geerdink, G. M., & Kluge, R. A. (2014). Chitosan applications pre- or postharvest prolong raspberry shelf-life quality. *Postharvest Biology and Technology*, *91*, 72-77. doi: 10.1016/j.postharvbio.2013.12.023

Xing, Y., Xu, Q., Li, X., Chen, C., Ma, L., Li, S., … Lin, H. (2016). *Chitosan-based coating with antimicrobial agents: Preparation, property, mechanism, and application effectiveness on fruits and vegetables*. Retrieved July 22, 2020, from https://www.hindawi.com/journals/ijps/2016/4851730/

Xu, J., & Zhang, S. (2015). Ethylene biosynthesis and regulation in plants. In C. K. Wen (Ed.), *Ethylene in plants* (pp. 1-25). Dordrecht, Netherlands: Springer Science+Business Media. doi: 10.1007/978-94-017-9484-8_1

Yamamoto, K., Amalia, A., Putri, S. P., Fukusaki, E., & Dwivany, F. M. (2018). Expression analysis of 1-aminocyclopropane-1-carboxylic acid oxidase genes in chitosan-coated banana. *HAYATI Journal of Biosciences*, *25*(1), 18-24. doi: 10.4308/hjb.25.1.18