Effects of Fruit Storage Chamber (FSC) and Chitosan Coating on Cavendish Banana (*Musa acuminata* AAA Group) *MaACO* and *MaACS1* Genes Expression

**F M Dwivany**1*, **A Aulina**1, and **A S Pratiwi**1

1Department of Biology, School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, West Java, Indonesia

*E-mail: fenny@sith.itb.ac.id*

**Abstract.** Banana is one of the most important fruit commodities and extensively used as raw material in local food industries. Unfortunately, the poor handling during postharvest storage often leads to sharp decrease in the qualities and quantities of banana fruits that are not acceptable to the market standards. Hence, this research aimed to investigate an alternative method for postharvest handling of banana, which is practical at low-cost. In this research, we tested the effects of chitosan coating and application of bamboo Fruit Storage Chamber (FSC) to delay ripening. Two kilograms of banana were placed in two tier FSC to determine its effect on fruit ripening process. Physical, physiological, sensory quality and ripening related (*MaACO* and *MaACS1*) genes expression levels were studied during nine days of the ripening process. The results showed that the total soluble solids (TSS), weight loss, and pulp to peel ratio of the control were higher than the FSC treated bananas. In addition, the level of *MaACO* and *MaACS1* genes expression in FSC treated bananas is also lower than in control. These results indicated that FSC treatment was able to delay the ripening of bananas by two days later than control.

**Keywords:** Fruit Storage Chamber, Gene expression, Chitosan, *MaACO*, *MaACS1*

1. **Introduction**

Bananas are considered as one of the most important crops in the world due to its potential to contribute to both food and economy sectors [1]. However, the utilization of banana fruit for food industry has not yet optimized. One of the main problems is post-harvest management, since banana is a climacteric fruit with short shelf life after ripening process commences. In addition, banana productions spread across this vast archipelago of Indonesia and urgently requires a suitable method of postharvest storage and transportation to improve banana-based trades.

In climacteric fruits, ethylene hormone plays a crucial role during ripening. The ethylene synthesis is regulated by two main enzymes: aminocyclopropane-1-carboxylic acid synthase (ACC synthase or ACS) and aminocyclopropane-1-carboxylic acid oxidase (ACC oxidase or ACO). The ACC synthase enzymes are encoded by *ACS* genes family and ACC oxidase by *ACO* genes family [2,3]. Ethylene biosynthesis process begins with the conversion of methionine (amino acid in cell) into S-adenosyl methionine (Adomet) catalyzed by adomet synthase. Adomet will then feed into ACC synthesis reaction, and in the presence of oxygen ACC will then be oxidised into ethylene, both processes occur...
in the cytosol. The activity of ACC Synthase is regulated by environment stresses, such as wounding, flooding, senescence, pathogen infection, and hormone signal by auxin and ABA [4].

Preliminary research was conducted to examine the effect of fruit storage chamber (FSC) application in extending the shelf life of banana [5]. The study reported that FSC treated banana fruits were edible until 9 days following ethylene treatment, whereas the control ones were only until 5 days. In addition, physiological analysis showed brix and pulp to peel ratios in FSC treated fruits were lower than in control. In this study, FSC treatment was also co-applied with chitosan coating of the fruits, which is predicted to reduce microorganism infection [6]. Molecular analysis was conducted together with physical and physiological analysis. The results of this study provides an alternative method for storing banana fruits at small and medium industries using at low cost.

2. Materials and methods

2.1. Fruit Storage Chamber (FSC)
The study was conducted in The School of Life Sciences and Technology, Institut Teknologi Bandung. The bamboo FSCs were placed in fumigated room at room temperature and unexposed to sunlight, in three replicates. Three different treatments were conducted: control without FSC, FSC in lower stack and FSC in upper stack. Two kilograms of bananas then were placed on each FSC and each FSC was stacked into two tier to determine the stacking effect on fruit ripening.

2.2. Banana fruit preparation
Banana fruits (Musa acuminate AAA Group cultivar) were supplied by PT. Sewu Segar Nusantara, Tangerang, Indonesia. All fruits were ethylene-treated, washed by detergent and wiped by 70% alcohol. The chitosan coating treatment was conducted using 1.25% (w/v) chitosan. The fruit sampling points were conducted on the day 0, 1, 3, 5, 7, and 9 of study.

2.3. Preparation of 1.25% chitosan
Chitosan was supplied by Biotech Surindo, Indonesia. This experiment was using chitosan powder food grade which was solubilized in glacial acetate acid (0.5%) and adjusted to pH 5.6 using 3M NaOH [5]. Coating was performed by dipping the tip of each finger in the prepared chitosan solution for two minutes and then air dried.

2.4. Physical and physiological analyses
Physical and physiological observation in this study was performed using method described by [8]. Physical and physiological analyses were conducted by observing banana peel color changes, iodine test, and measurement of pulp to peel ratio, weight loss, and total soluble solids. Peel discoloration was documented with Canon A2500 digital camera. The SPSS one-way ANOVA statistical analysis was performed to measure mean and significant differences between two or more independent groups.

2.5. Sensory quality test
The sensory quality tests was performed as described by [9] (with some modifications). The test was conducted to determine the texture, aroma, taste, and color of banana against 15 respondents in three repetitions. The 7 numeric scales to select by respondents represent 1 for the extremely dislikes up to scale 7 which respondents feel strongly like the taste. The SPSS one-way ANOVA statistical analysis was performed to determine the average of each measured parameter.

2.6. Molecular analysis
Total RNA was isolated using modified methods of [10]. The cDNA synthesis then was done using iScript™cDNA Synthesis (Biorad catalog number: 170-8890). Amplification of cDNA samples were done by PCR Thermalcycler machine using Promega master mix kit (catalog number: M7122). The gene specific primers for MaACO and MaACSI as well as MaGAPDH were used as mentioned by[7].
PCR result was then confirmed by electrophoresis with agarose gel 1% (w/v) in TAE 1x and visualized under UV light. The visualized band then was quantified by Image J (http://www.imagej.net). The result was then calculated as relative MaACS or MaACO1 expressions which were normalized to MaGAPDH gene expression (as reference gene).

3. Results

3.1. Physical analysis
Many criterions are assessed to detect the ripening process in banana such as the physical and physiological condition and the study of the ripening related genes. The result of observation on peel color changes during ripening on different treatments is shown in figure 1. The color changes were obvious during ripening stages especially on the 7th and 9th day of observation and between the control and treatments (FSC and chitosan coating in both upper and lower stacks). The changes in peel color were then confirmed by iodine test and the results are also shown in figure 1. The results verified that iodine exposed surface area decreased during ripening. However, it was difficult to observe iodine test result on over ripe fruits from day 0 to day 9 due to its cell integrity.

Figure 1. Change in color of banana peel and the results of iodine test during fruit ripening.

3.2. Physiological analyses
3.2.1. Total soluble solid. Sugars are found the most abundance soluble compounds in the pulp. Among with another compounds such as vitamin C, acids, amino acids, and some pectin, these compounds form the soluble solids in the flesh of the fruit. Total soluble solids can be an index to screen the ripening in the fruit since the sugar content will also increase as they start to ripen. The total soluble solid (TSS) was calculated by unit of degrees Brix. The TSS analysis showed that in general, control fruits displayed higher values compared to fruits from both treatment groups (figure 2).
3.2.2. Weight loss percentage. Weight loss of control group increased gradually since day 5 and had higher values than both of FSC with chitosan treatments (figure 3). Statistical analysis using one-way ANOVA ($P < 0.05$) showed that each treatment had significant differences. This may indicate the starch degradation occurs faster in the control group than the treatment from both tier which affect to the weight loss percentage.

3.2.3. Pulp to peel ratio. Pulp to peel ratio changes during the ripening is one of the most significant ripening indicators [7]. The results of banana pulp to peel ratio analysis in all treatments showed an increase trend from day 0 to day 9 and the control group had a higher value than other treatments (figure 4). The statistical analysis using one-way ANOVA ($P < 0.05$) showed that control and other treatments differed significantly.
3.3. Molecular analysis
Semi quantitative genes expression analysis was performed by RT-PCR using *MaGAPDH* as the housekeeping gene. Both *MaACS1* and *MaACO* genes expression were higher in control group compared to the other treatments (figure 5). However, statistical analysis showed that there was no significant difference between the three treatments (P>0.05).

3.4. Sensory analysis
The results of sensory quality test to 15 respondents in 3 times repetitions showed the tendency of respondents’ favorite on texture, flavor, aroma, and color of banana fruits. In general, respondents liked the fruits from both lower and upper stacks which were placed in FSC and treated with chitosan coating better than the control ones (figure 6). In control, respondents tended to dislike the texture, color, and aroma of the fruit. However, both from the treatment group, respondents tended to like every parameters measured. Based on the fruit appearance, all of the bananas from the treated group were in a good condition which is dissimilar to the appearance from the control group.
4. Discussion

Ripening is a complex process which involves numerous metabolical pathway in the cell. This pathway may affect the physical, physiological, and molecular condition of the fruit which is interconnected. Color changing in banana fruit is the major transformation occurs during ripening. Banana undergoes discoloration from green to yellow and eventually brown due to the degradation of chlorophyll by the chlorophyllase enzyme. Meanwhile, the yellow coloration on the banana peel is a consequence of the increasing amount of carotenoid in the cell [4]. The discoloration of banana peel which occurred faster in control than the treatment may indicate that storage treatment may affect the delaying of the ripening process. Based on the result, there were no significant differences between the upper and bottom tier. To investigate further, iodine test was performed to determine the starch content quantitatively from the banana samples. Starch and iodine reaction give the black or dark purple coloration and the color will decrease during maturity. The data showed that FSC storage and chitosan coating treatment indicates a delayed ripening process.

In addition, TSS value will increase along with the maturity stage in banana due to the rise of sugar content in the fruit. Based on the TSS result, the upper stack treatment did not differ significantly from lower stack treatment based on the results of one-way ANOVA statistical analysis. The value of FSC treatment differed significantly from the control group (P <0.05) on day 3 until 9. The total soluble solid values changed during ripening process and increased as the hydrolysis process converted starch into sugars [8]. The value of weight loss percentage also showed the indication of the delaying ripening process in both treatments, upper and lower storage FSC treated with chitosan. Weight loss percentage and pulp to peel ratio corresponds to each other during ripening. This result showed that respiration and transpiration in control group were higher than in the treatment ones. It is known that respiration and transpiration processes are controlled by the stomata guard cells [4]. The increase in banana pulp to peel ratio was the result of starch hydrolysis and the osmotic process of water, leading to the rind of banana fruit pulp [8]. The values of three physiological analyses above indicate that treatment with FSC and chitosan was able to delay fruit ripening process when compared to control.

Chitosan coating might help the fruits to retain water content during ripening stage. In addition, bamboo FSC could play a role in reducing gas exchanges, especially O2 and CO2, around fruit which results in reducing respiration and ripening processes.

Generally, ethylene plays a big role in ripening process [4]. Both MaACS1 and MaACO genes are keys in the synthesis of ethylene, where increasing gene expression might correlate with higher ethylene production. Expression of ACS gene is influenced by ripening process itself whereas ACC oxidase is also influenced by the availability of oxygen [4,11]. The results suggested that FSC might reduce the air flow that potentially altered ethylene synthesis and ripening process.
The sensory analysis from respondents was also conducted to investigate the preferences of the consumer. Bananas from treatment groups were preferable than the control group due to several main criterions which contributes to the willingness to consume the fruit. Therefore, based on these analyses, FSC storage and chitosan coating might be capable to elongate the banana shelf-life by delaying its ripening process. In addition, stacking the FSC during the storage did not give any significant differences to the banana which can be applicable to the banana-based food industries.

5. Conclusion
The results of physical, physiological, sensory quality test, and molecular analysis confirm the influence of FSC application towards banana ripening process. However, there was no significant result observed between stacking of two FSC arrangements. In this study, the arrangement of FSC was performed to simulate real storage condition. We suggest that further studies with more stacking numbers (>2) is needed to evaluate the capability of FSC in delaying banana ripening as well as transcriptomics study to know more comprehensive correlation between fruit ripening with gene expression profiles.

Acknowledgements
This study was a Banana Group (Institut Teknologi Bandung, Indonesia) Research Project and conducted with the financial support of MP3EI-Ristek DIKTI for Fenny M. Dwivany. The authors would like to thank to all Banana group team members and PT. Sewu Segar Nusantara supports during this study.

References
[1] Langhe E De, Vrydaghs L, De P, Perrier X and Denham T 2009Ethnobotany Research and Applications 7165–77
[2] Liu X, Shiomi S, Nakatsuka A, Kubo Y, Nakamura R and Inaba A 1999 Plant Physiol
1211257–65
[3] Huang PL, Do YY, Huang FC, Thay TS and Chang TW 1997 Biochem. Mol. Biol. Int. 41 941–50
[4] Taiz L and Zeiger E 2010 Plant Physiology (Sunderland: Sinauer Associates)
[5] Pratiwi A, Dwivany FM, Larasati D, Islamia HC and Martien R 2015 AIP Conf. Proc.1677
[6] Dutta PK, Tripathi S, Mehrotra GK, Dutta J 2009 Food Chemistry 114 1173–1182
[7] Dadzie BK and Orchard JE 1997Routine post-harvest screening of banana/plantain hybrids: criteria and methods (Montpellier: INIBAP Technical Guidelines)
[8] Peryam DR 1998The 9-Point Hedonic Scale (Chicago: Peryam & Kroll Research Corporation)p 1–10
[9] Cordeiro MCR, Silva SM, de Oliveira-Filho EC, de Miranda Z de JG, Aquino F de G and Fragoso R da R 2008II Simpósio Int. Savanas Trop. 1–6
[10] Alexander L and Grierson D 2002 J. Exp. Bot. 53 2039–55