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Bamboo Fruit Storage Chamber (FSC) Equipped with Ethylene-Degrading Manganese Doped Titanium Oxide Nanomaterial as Storage for Banana (*Musa acuminata*)

(Ruang Penyimpanan Buah Buluh (FSC) Dilengkapi dengan Bahan Nano Titanium Oksida Didop Mangan Etilena sebagai Penyimpanan Pisang (*Musa acuminata*)

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ABSTRACT

As a climacteric fruit, banana undergoes rapid ripening induced by the hormone ethylene, which is produced by autocatalytic reactions. Titanium dioxide is a photocatalytic compound with the ability to degrade ethylene to water and carbon dioxide. This compound can be used to control the concentration of ethylene inside storage chambers to delay the ripening process of bananas in storage. A passive modified atmosphere is another method to delay ripening by using storage spaces with limited air flow. This study attempts to investigate the performance of TiO_2-Mn and bamboo fruit storage chamber (FSC) to delay the ripening of bananas by measuring characteristic physiological changes for 7 days which included ethylene accumulation in storage space, rate of ethylene production, rate of respiration, starch content, and soluble sugar content. The results show that the use of FSC in combination with TiO_2-Mn can be used to delay the ripening of bananas. This study also investigated the effect of volumetric occupation to the efficacy of FSC by varying the number of banana fingers in storage and varying the volume of the chamber. While the volume of the FSC did not produce a significant difference in performance, the number of bananas stored in each FSC greatly influenced the delay-ripening ability of FSC with TiO_2-Mn. At the end of the study, a profile plotted with MATLAB is presented to show the relationship of ethylene concentration in FSC in respect to storage time and number of fingers stored.

Keywords: Cavendish banana; delay ripening; ethylene; fruit storage chamber; TiO_2

ABSTRAK

Sebagai buah klimakterik, pisang mengalami kematangan yang cepat disebabkan oleh hormon etilena. Etilena dihasilkan oleh pisang semasa masak melalui tindak balas autokatalitik. Titanium dioksida adalah sebatian fotokatalitik dengan keupayaan memecahkan etilena menjadi air dan karbon dioksida. Sebatian ini boleh digunakan untuk mengawal kepekatan etilena di dalam ruang simpanan untuk melambatkan proses masak pisang dalam simpanan. Atmosfera terubah suai pasif adalah kaedah lain untuk melambatkan pematangan pisang. Ia boleh dicapai dengan menyimpan pisang di dalam ruang yang mengehadkan aliran udara. Kajian ini cuba menyelidik kebolehan TiO_2-Mn dan ruang penyimpanan buah (FSC) untuk melambatkan pematangan pisang dengan mengukur perubahan fisiologi selama 7 hari yang merangkumi kepekatan etilena dalam FSC, kadar pengeluaran etilena, kadar respirasi, kandungan kanji dan kandungan gula larut. Hasil kajian ini menunjukkan bahawa penggunaan FSC dalam gabungan dengan TiO_2-Mn boleh digunakan untuk melambatkan pematangan pisang. Kajian ini juga menilai pengaruh ruang isi padu kepada kecekapan FSC dengan mempelbagaikan jumlah pisang dalam simpanan dan mempelbagaikan saiz ruang. Walaupun volum FSC tidak menghasilkan perbezaan hasil yang ketara, jumlah pisang yang disimpan dalam setiap FSC sangat mempengaruhi keupayaan FSC dengan TiO_2-Mn dalam melambatkan pematangan pisang. Pada akhir kajian, profil yang diplot dengan MATLAB dibentangkan untuk menunjukkan hubungan kepekatan etilena dalam FSC berkenaan dengan masa penyimpanan dan bilangan pisang yang disimpan.

Kata kunci: Etilena; pisang Cavendish; melambatkan pematangan; ruang penyimpanan buah; TiO_2

Introduction

Cavendish banana is the major variety of banana sold in the market. However, the fruit is highly perishable (Paggi & Spreen 2003). Distributions and sales of Cavendish banana often involve a long supply chain. This often cause problems such as the decay of bananas before reaching customers due to rapid ripening. The ripening of banana starts with induction by ethylene gas, followed by the cascade of signals resulting in physio-chemical changes in the fruit. These changes require a large amount of energy that is obtained from the respiratory climacteric (Chesworth et al. 1998). Limiting exposure to ethylene gas and the availability of oxygen are both alternatives to slow down the rate of ripening.

Ethylene is produced by banana in an autocatalytic pattern (Klee et al. 2004), which means that the reduction of ethylene concentration could decrease the subsequent production of ethylene. Limiting the exposure to ethylene gas could be achieved by cutting this cycle. Alternatives to cutting this cycle without having to manipulate the internal production would be to decrease the sensitivity of ethylene receptors (Ooraikul & Stiles 1991) or breaking down the ethylene already present by using a catalyst.

One of the most promising materials to break down ethylene is titanium dioxide (Wills & Golding 2016). The activation of titanium dioxide as a photocatalyst requires energy in the form of photons; with sufficient levels of energy achieved by utilising UV light. However, the use of UV light has higher risks and costs compared to visible light. This could be solved by doping the titanium dioxide with a transition metal to increase its performance and enable it to be activated by visible light (Lee 2010). Manganese is one of the potential elements to achieve this, as it had been shown to produce a red-shift to titanium oxide's optical absorption edges (Momeni et al. 2015).

Modified atmosphere is another way to delay the ripening of banana by limiting the availability of oxygen, thus slowing respiration. Modified atmosphere can be achieved actively by storing the fruit inside a package and flushing the package with a specific gas mixture or passively by the depletion of oxygen by the respiring fruit stored inside the package (Sen et al. 2012). This study used a passive modified atmosphere using a Fruit Storage Chamber (FSC) made of bamboo. Bamboo has been chosen for its inert characteristic, abundance, and rapid regeneration in Indonesia (Pratiwi et al. 2015) therefore, ensuring that the manufacture of this chamber is sustainable.

In this study, a novel storage method is proposed using titanium dioxide doped with manganese combined with bamboo-based FSC. The objective of this study was to investigate the ability of this storage method to delay the ripening of bananas by evaluating the physiological parameters of ripening over a period of one week and comparing the results against a control group stored in open space. This study also aims to evaluate the performance of this storage method in respect to the quantity of bananas stored in one space and the volume of the chamber. The ripening parameters used in this study are concentration of ethylene in storage space, ethylene production rate, respiration rate, soluble sugar content, and starch content.

Materials and Methods

Materials

Cavendish banana was provided by PT Sewu Segar Nusantara at 9-weeks after flowering. The bananas were subjected to ripening induction using ethylene gas prior to testing. The bananas were sorted to obtain a uniform physiological stage. Titanium dioxide doped with manganese was obtained from Inorganic Chemistry Laboratory, FMIPA ITB. The material was manually coated on a square glass plate and heated to dry. The coating was done with a grid pattern across an area of 144 cm^2 and a thickness of 0.1 - 0.5 mm.

Bamboo FSCs were obtained from a local craftsman at Sumedang Regency. The interior surface of the chambers was lined with thick craft paper to further limit gas exchange. The dimensions of the fruit chambers for the first and second experiment were 30 × 30 × 15 cm. The third experiment evaluating the volume of chambers used two sizes of FSC. The dimension of the small FSC was 17 × 17 × 10 cm. The dimension of the large FSC was 30 × 30 × 15 cm. The FSCs used for the third experiment were not fitted with TiO_2-Mn plates.

RIPENING PROCESS

The ripening process was divided into groups of three fingers to simulate the storage of bananas in bunches. In the first experiment, bananas were stored in different storage methods to investigate the effect of using FSC and TiO_2-Mn. The setup of the different storage methods was a) in open space, b) inside an FSC without TiO_2-Mn plate (Figure 1(A)), c) underneath an illuminated plate of TiO_2-Mn with a 10 cm space between the banana and the plate (Figure 1(B)), and d) inside an FSC equipped with TiO_2-Mn plate (Figure 1(C)). The FSC without TiO_2-Mn plate has a blank glass plate in its place that is uncoated with TiO_2-Mn. Each storage space was illuminated by a 3-watt LED light bulb. The bananas stored in open space (a) would be referenced as the control group. All of the bananas were stored in a dark room and arranged side by side (Figure 1(D)).

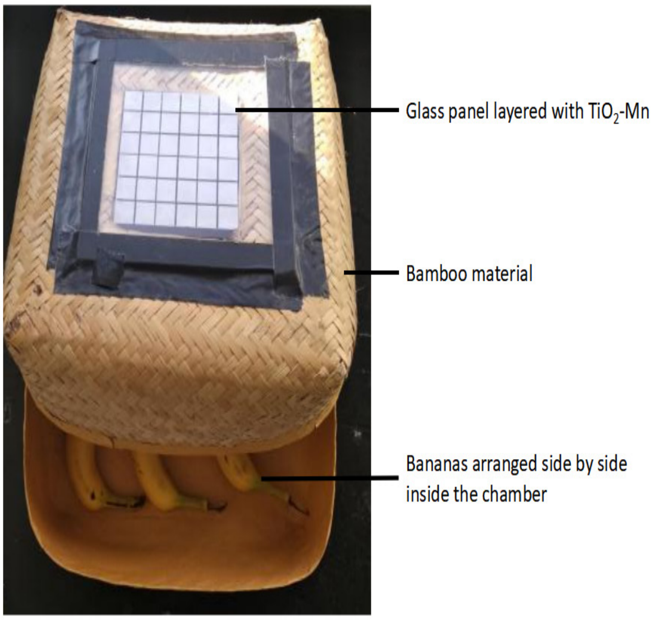


FIGURE 1. Storage setups (A) FSC without TiO_2-Mn plate; (B) TiO_2-Mn without FSC; (C) FSC equipped with TiO_2-Mn; (D) banana arrangement during storage

The second experiment was used to investigate the efficacy of each storage space in respect to number of banana fingers stored. The bananas were stored in FSCs equipped with TiO_2-Mn in groups of six fingers to simulate a hand of bananas, groups of one finger to simulate a singular finger, and groups of three fingers as the middle of the range of numbers. Each FSC was placed on an enclosed tray illuminated by 3-watt LED light bulbs. The third experiment was performed to investigate the effect of FSC volume to the ripening of bananas. In the third experiment, the bananas were stored in groups of three fingers.

Sampling was carried out at one, three, five, and seven day(s) of storage. All samples were stored in a closed and temperature-regulated room with temperatures kept at 22-24 °C.

MEASUREMENT OF ETHYLENE CONCENTRATION

The concentration of ethylene in the storage space was measured to determine the ethylene degrading capability of the TiO_2-equipped FSC. This measurement was only carried out to banana samples stored in FSC because the chamber provides an enclosure for the ethylene to accumulate. Measurement was carried out using Ethylene Spy ES-900 Fruit Control Equipment in accordance with the manufacturer's instructions. Measurements were taken in triplicates.

ETHYLENE PRODUCTION AND RESPIRATION RATE MEASUREMENT

In climacteric fruits, an increase of ethylene production and respiration rate occur during ripening, culminating in a climacteric peak (Sen et al. 2012). By measuring ethylene production and respiration rate in respect to time, the ripening process of the banana samples can be evaluated. Ethylene production and respiration rate were measured inside glass containers using a closed system method as described by Mendoza et al. (2016) with modifications. The sampled banana was displaced from its storage space and then kept inside the glass container for 10 min. After 10 min, the amount of CO_2 produced by the banana in the chamber was measured using Lutron GCH-2018 CO_2 meter to calculate the respiration rate. Using the same method, Ethylene production was measured using Ethylene Spy ES-900 Fruit Control Equipment. Measurements were taken in triplicates. The measurement of ethylene production produces the rate of ethylene produced by the banana after removal from the storage chamber, which reflects the banana's stage of ripeness; whereas the concentration of ethylene measures the amount of ethylene accumulated in a storage chamber, which reflects the ability of TiO_2-Mn in degrading ethylene across the span of the storage period.

DETERMINATION OF STARCH AND SUGAR CONTENT

Unripe bananas store a large number of starches, which degrade into soluble sugars over the course of ripening (Do Nascimento et al. 2006). In this study, the starch content of bananas was measured upon arrival (day 0) and on the 7th day of sampling to compare the difference between the starch content of bananas before and after ripening. The measurements were carried out using Luff Schoorl's method, modified from Balthrop et al. (2011). Total soluble solids were measured every two days using an Atago digital refractometer and the Brix value was used to estimate the total soluble sugar of the banana sample. The results were calculated from a calibration curve and presented as percent equivalent sucrose. Measurements were taken in triplicates.

STATISTICAL ANALYSIS

Data are presented as average values of triplicate measurements (±SE). One way analysis of variance was performed to compare the performance of different storage conditions to the ethylene production rate and degradation of starch in bananas (p-value < 0.05).

RESULTS AND DISCUSSION

EFFECT OF TiO_2-MN ON THE PHYSICOCHEMICAL CHANGES DURING BANANA RIPENING: ETHYLENE PRODUCTION AND CONCENTRATION IN FRUIT STORAGE CHAMBER

Ethylene production rates differed between samples of different storage methods (Figure 2). The production rate of ethylene in the bananas stored in FSC without TiO_2-Mn was lower significantly on the 7th day compared to the bananas stored in open space ($p = 2.55 \times 10^{-8}$). This might be due to the lower availability of oxygen inside the FSC after 7 days of respiration in the space in which airflow was restricted. A decrease of oxygen in a passive modified atmosphere had previously been observed by Siriwardana et al. (2018) using a polymer-film package to store 5 'Embul' banana fingers for 7 days and by Madan et al. (2014) using HDPE to store Robusta banana for 9 days.

The reduced oxygen level in this study might lower the sensitivity of ethylene receptors and inhibit the activity of 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO), the plant hormone involved in the final formation of the ethylene compound, thus slowing down ethylene production (Valero & Serrano 2010). The rate of ethylene production from bananas stored in FSC equipped with TiO_2-Mn exhibited an even more significant decrease ($p < 0.000001$), starting from the 3rd day to the 7th day. This low rate could be caused by the prevention of an autocatalytic ethylene production due to TiO_2-Mn's role in keeping the ethylene levels low in the FSC for a longer period of time, as can be seen in Figure 3. However, the production of ethylene from the sample stored underneath a plate layered with TiO_2-Mn did not show any difference from the control group, in which the bananas were not stored in an FSC. These results showed that the use of TiO_2-Mn for delaying ethylene climacteric peak required a closed space. A closed space would increase the chance of ethylene, as reactant, to come into contact with the layer of TiO_2-Mn as catalyst of the reaction.

The ethylene concentrations in FSC with and without TiO_2-Mn were the same up to the third day (Figure 3).

However, the concentration on the 5th day increased drastically in the FSC without TiO_2-Mn. This pattern was similar to the pattern exhibited by bananas stored in micro perforated polyethylene (Pesis et al. 2005), although the increase occurred sooner in this experiment. The higher ethylene concentration was consistent with the fact that the samples in FSC without TiO_2-Mn produced ethylene at a higher rate than the ones stored in FSC with TiO_2-Mn (Figure 2). The FSC equipped with TiO_2-Mn managed to keep the ethylene concentration low even up to the 5th day. On the 7th day, however, the concentration increased to the same level as the FSC without TiO_2-Mn (Figure 3). Results from the second study using a larger FSC also showed that the average level of ethylene was slightly lower when three fingers of bananas were stored inside a large FSC, compared to a small FSC (Figure 7(b)).

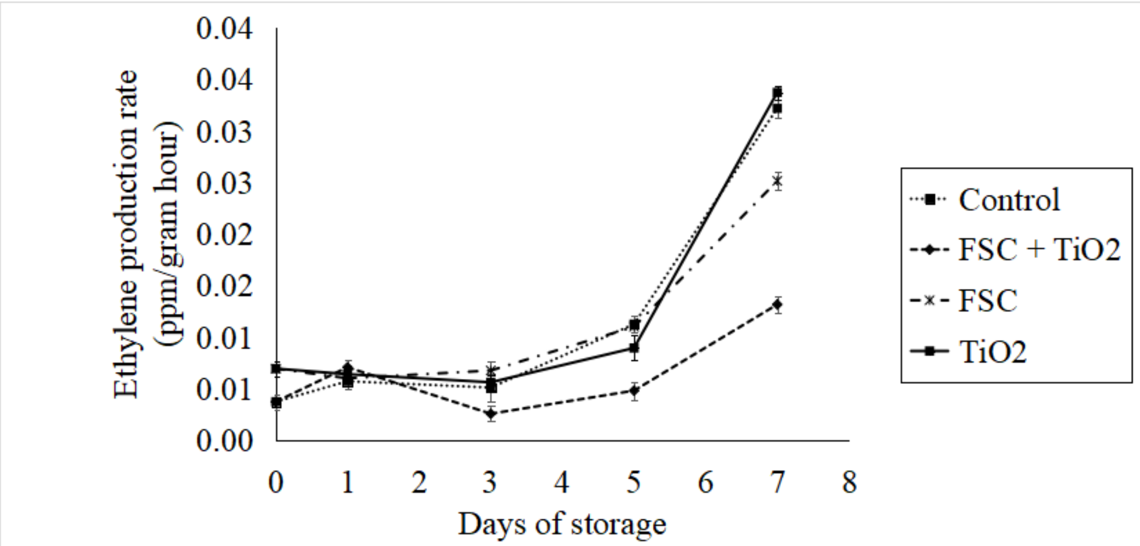


FIGURE 2. Ethylene production rates of bananas with different storage methods

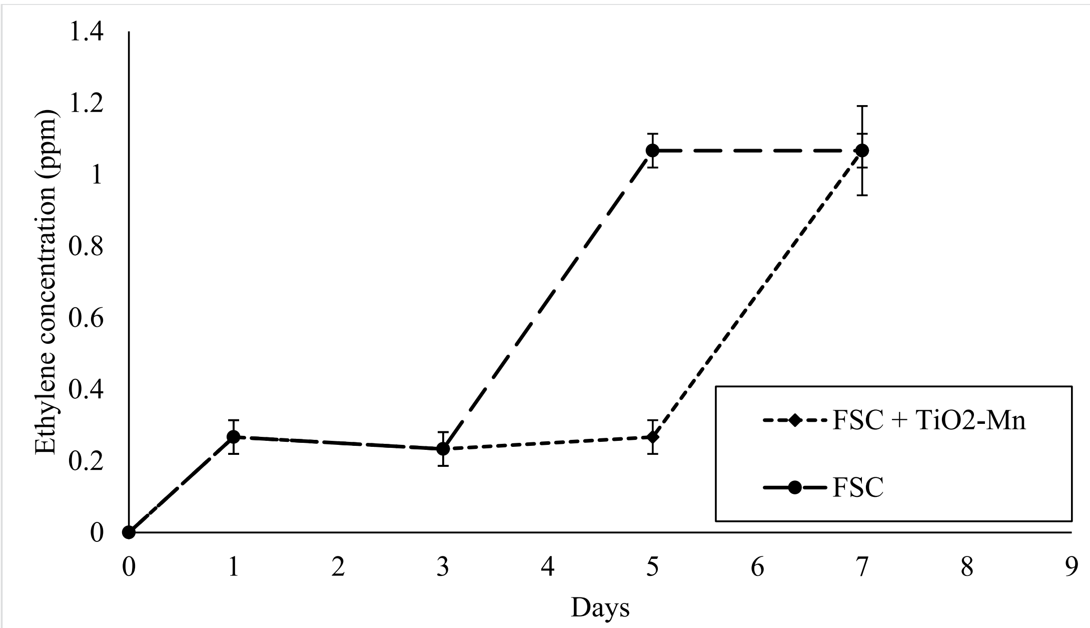


FIGURE 3. Concentration of ethylene in FSC with and without TiO_2-Mn

The increase in ethylene concentrations might be due to the accumulation of the increasing rate of ethylene production as a result of the positive feedback mechanism regulating the production of ethylene (Mauseth 2011). Bananas inside the FSC were in constant exposure to the ethylene they produced, triggering the autocatalytic reaction, explaining the drastic increase exhibited by both samples. The difference was the time needed for the ethylene concentration reached the maximum value for each sample. The ethylene concentration of the chamber with TiO_2-Mn was sufficiently suppressed until day 5. However, on day 7, the ethylene production managed to surpass the degradation reaction. This was due to the inability of TiO_2 to completely degrade ethylene. Kumar et al. (2005) showed that the efficiency of ethylene degradation catalysed by TiO_2 using visible light could only reach 8.7-11.9%. Doping with manganese showed an increase in degradation performance in UV light, but the ethylene degradation still did not reach 100% (Kemp & McIntyre 2006). This accumulation of incomplete degradation of ethylene was what caused the end concentration in the FSC to be the same, with or without TiO_2-Mn. The results showed the ability of TiO_2-Mn to control ethylene levels, albeit with a limit.

RESPIRATION RATES

Cellular aerobic respiration needs oxygen (O_2) to produce carbon dioxide (CO_2) and water vapor (H_2O) as well as ATP and an increase in temperature. Respiration rates for all samples reached their peaks before the 7th day, except for the sample stored in FSC equipped with TiO_2-Mn (Figure 4).

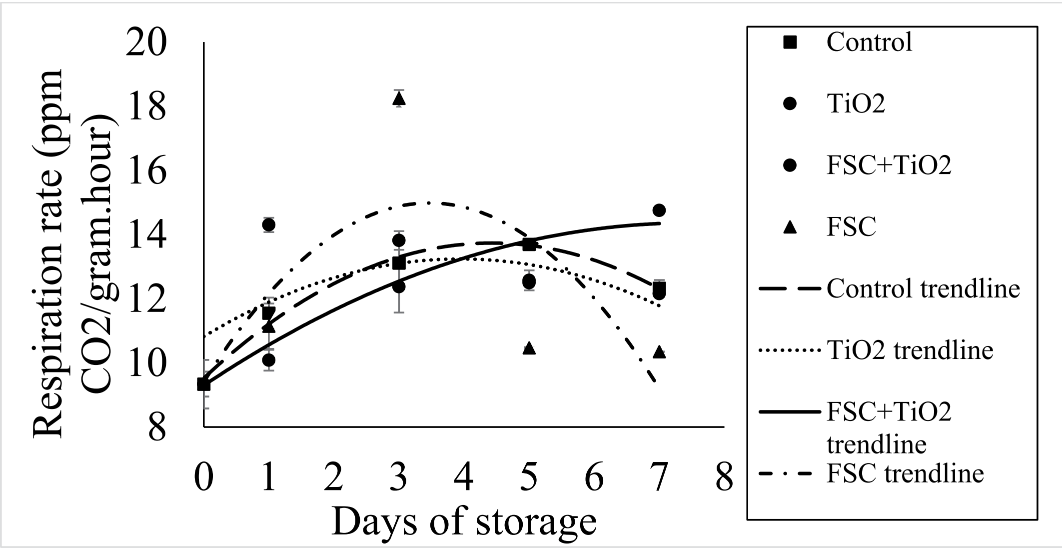


FIGURE 4. Respiration rate of bananas during ripening in different storage methods

The cause of this difference was possibly due to the low concentration of ethylene in the FSC as a result of degradation by TiO_2-Mn. The low concentration of ethylene would in turn cause the delay in reaching the peak of respiration. The use of either FSC or TiO_2-Mn separately neither yielded a delay in respiratory climacteric nor a lower overall respiration rate (Figure 4). The effect of TiO_2-Mn in the open air was not enough to influence the ripening of banana, as was shown previously from the rate of ethylene production (Figure 2). On the other hand, storage in FSC without TiO_2-Mn resulted in a rapidly declining rate of respiration. This might be due to the limited oxygen supply resulting in anaerobic respiration (Valero & Serrano 2010). The same phenomenon was also observed when smaller chambers were used to store three fingers of bananas compared to larger FSCs (Figure 7(b)). Therefore, the ratio between the number of stored banana and chamber size was crucial for respiration, which affected ethylene production and hence ripening.

STARCH AND TOTAL SOLUBLE SUGAR CONTENT

One of the physiological changes occurring during ripening is the conversion of starch into simple sugars (Abeles et al. 1992). The starch content before ripening accounted for 30.99% of the total fresh weight (Figure 5). This value was close to the amount determined at a previous study by Cordenunsi and Lajolo (1995), which was 25%.

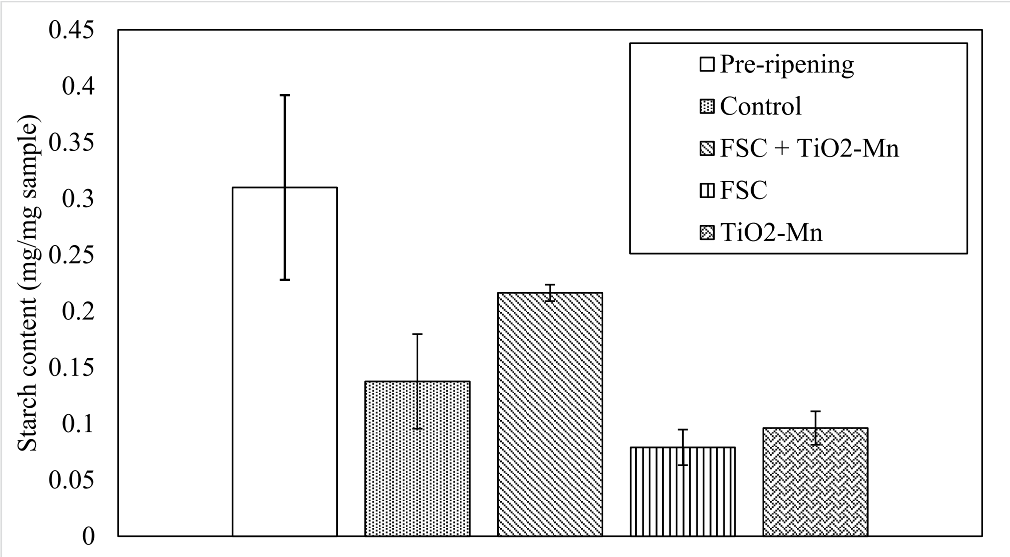


FIGURE 5. Starch content of banana before ripening and at day 7 of storage after ripening in various storage conditions

Bananas stored in FSC equipped with TiO_2-Mn had an average starch content of 21.6% by the end of the 7-day storage time, which was significantly higher than the other samples ($p = 0.0019$). Storage in either FSC or TiO_2-Mn separately produced a lower content of starch, even compared to the control sample stored in open space. The results supported the conclusions that in order for the TiO_2-Mn to perform, it must be combined with FSC. Total soluble sugars of all the treatments showed a similar pattern with a slight difference on the 1st to 5th day, and then converged on the 7th day (Figure 6).

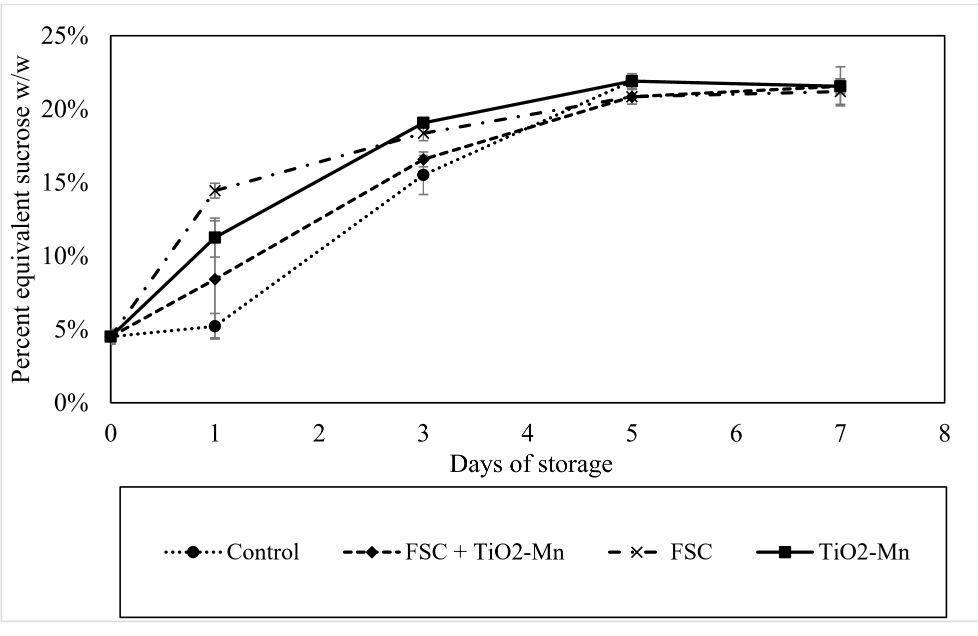


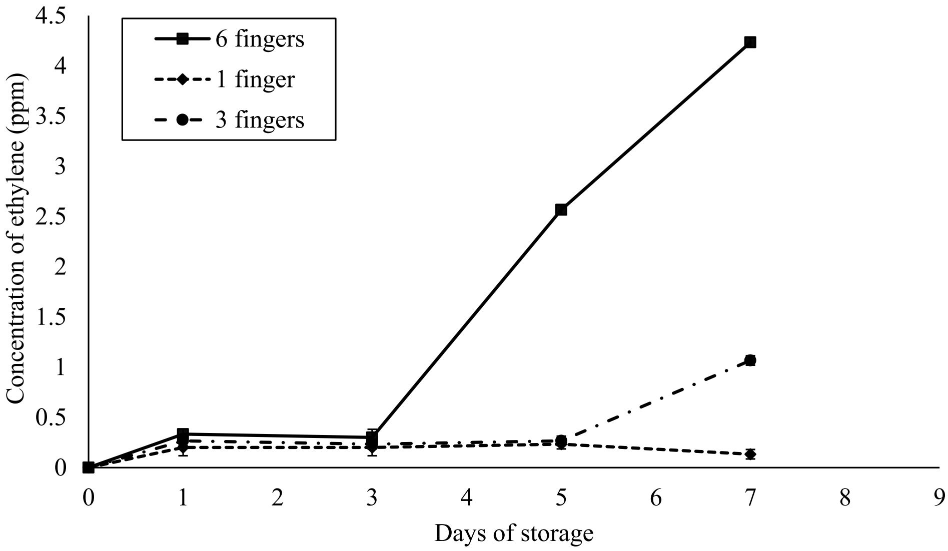
FIGURE 6. Total soluble sugars of bananas during ripening in different storage methods

The amount of total soluble sugars on the 7th day of treatment was around 21% sucrose equivalent. This result was like previous research, which was 23% (Marriott et al. 1981). The pattern of increasing soluble sugars was not always matched with the decrease in starch, which could be caused by sugar being used up for respiration.

EFFECT OF NUMBER OF BANANA FINGERS STORED IN FRUIT STORAGE CHAMBER EQUIPPED WITH TiO_2-MN

The second experiment assessed the effect of the bamboo FSC's volumetric space to delay ripening. The assessment was done by varying the number of banana fingers stored in the FSC. Increasing the number of bananas stored within the FSC will increase the rate of ethylene production. The aim of this assessment is to evaluate the profile of ethylene concentration over the period of storage time, which will show the capacity of TiO_2-Mn in maintaining ethylene concentration with varying production rates.

(A)



(B)

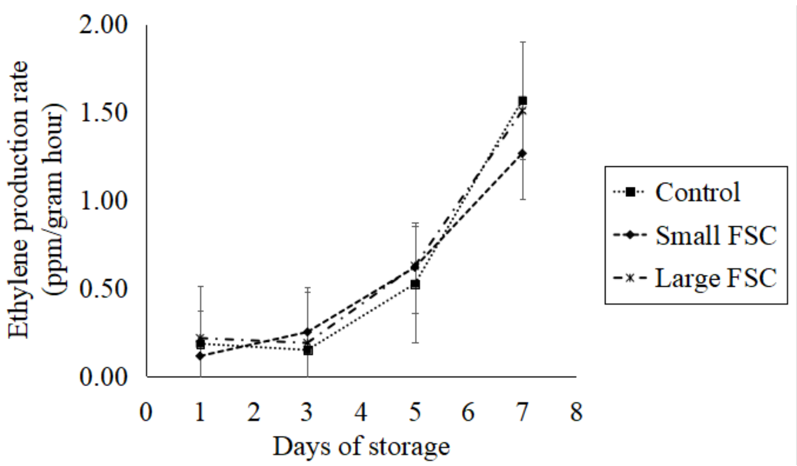


FIGURE 7. Ethylene concentration inside Fruit Storage Chamber (A) with varying numbers of banana finger; (B) inside a small and large Fruit Storage Chamber with three banana fingers

The results of the effect of varying the numbers of banana on the profile of ethylene concentration in FSC for seven days of treatment are shown in Figure 7(a). Generally, the ethylene concentration in the FSC showed an increasing trend as storage time and the number of bananas increased. During the first three days of treatment, the increase in ethylene concentration was similar for all variations. A significant increase was observed after the 3rd day for the six banana fingers treatment that continued until the 7th day. On the other hand, the one and three banana-fingers treatment remained constant up to the 5th day. After the 5th day, the three banana fingers treatment showed an increase, while the one-banana finger treatment showed a decrease in ethylene concentration. These results showed that in this research, the capability of TiO_2-Mn in delaying the ripening of banana for seven days of storage was only effective for having up to three-banana fingers in one storage space. When more bananas were stored, the capability of TiO_2-Mn in degrading ethylene was slower than the production rate of ethylene of the bananas. Therefore, there would be an accumulation of ethylene inside FSC, and ethylene would give more induction for ripening of the bananas inside. To increase the degradation capability, there needed to be a change either with an increase of storage space or an increase in the surface area of the TiO_2-Mn layer.

The third experiment was performed to test if a larger FSC would help disperse the ethylene, thus preventing autocatalytic production of it. Using two variations of FSC sizes, the result (Figure 7(b)) showed that the volume of the chamber only slightly affected the concentration of ethylene in that space ($p > 0.05$). In general, ethylene concentration increased from the 1st to 7th day, and the concentrations were similar during the first three days of treatment. Ethylene concentration in the small chamber had a higher increase in the 5th day compared to the large chamber, though not significant, which might be due to an increase in ethylene production related to ripening as well as to stress related to being in a tight chamber, as bananas have been known to generate heat during respiration (Jedermann et al. 2014). However, the average rate of ethylene production in the small chamber was slightly lower on the 7th day than the rate observed in the large chamber. The small chamber likely had fewer oxygen molecules at the beginning of storage due to its smaller volumetric space, which, with the same amount of bananas, would deplete faster than the oxygen in the large chamber. An oxygen deficit atmosphere produces lower productions of ethylene, as observed by Imahori et al. (2013).

A three-dimensional profile had been constructed to visualise the relationship of ethylene concentration in a storage chamber as a function of storage time and number of banana fingers stored. The curve was made using the curve fitting tool in MATLAB (Figure 8).

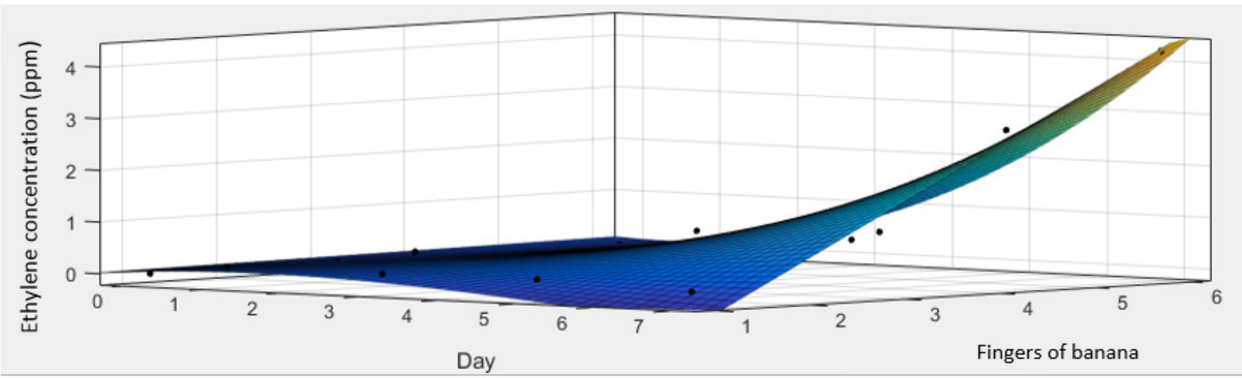


FIGURE 8. Profile of ethylene concentration in a banana storage chamber in respect to days and number of fingers stored

The curve followed a polynomial equation with x as storage time, y as banana fingers stored, and z as ethylene concentration in ppm. The curve fitted with the data well with an R-square value of 0.9356.

$$f(x,y) = 0.0606 + 0.134x + 0.001649y - 0.05405x^2 - 0.0296xy + 0.001719x^3 + 0.02189x^2y \quad (1)$$

Equation (1) would be able to predict the ethylene levels accumulated in a storage chamber after a certain storage time and stored with a certain number of banana fingers. The equation would also be able to predict how long a group of bananas would reach their climacteric peak.

Figure 8 shows that although FSC combined with TiO_2-Mn could delay the ripening of bananas, this ability is limited by the number of bananas stored. The 30 × 30 × 15 cm sized FSC combined with 144 cm^2 TiO_2-Mn could only keep ethylene levels low for storage of less than 3 bananas. Since it was found that the volumetric space did not significantly affect the delay-ripening performance of passive modified atmosphere using bamboo FSC (Figure 7(b)), a proposed solution might be to increase the surface area of the TiO_2-Mn plates to keep ethylene levels low for larger groups of bananas.

CONCLUSIONS

This study supported the possibility of ethylene production inhibition and delay of ripening in bananas using a combination of TiO_2-Mn as photocatalyst activated using visible light and storage in a bamboo fruit storage chamber (FSC). Physiological changes marking the ripening process in bananas, i.e., climacteric respiration and degradation of starch appeared to take effect at a later time in samples subjected to storage inside FSC equipped with TiO_2-Mn. However, the performance of TiO_2-Mn came with a limit; ethylene accumulation from more than three bananas would be too much for the material to degrade, and even when only three bananas were stored, the degradation became inefficient on the 7th day. A curve based on experimental data was obtained and yielded an equation that would enable calculations of ethylene accumulation in respect to number of banana fingers stored and storage time. Further studies should explore the possibility of scaling up this storage system using improved TiO_2-Mn in order to increase contact of ethylene to its surface area.

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