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Prediction of Ethylene Production Rate of Barangan Banana Using Kinetic Enzymatic Michaelis-Menten

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Abstract. MM is a model that describes the rate of reaction of product ethylene towards the substrate concentration and MMC is an enzymatic kinetics model to see the effect of the increase of O₂ on the rate of reaction. The objective of the study was to estimate ethylene production rate in bananas by applying the Michaelis-Menten enzymatic kinetics equation. Barangan Banana with harvest age 11 and 10 weeks were measured respiration rate with continuous gas analyzer in temperature 10, 20, and 28 °C with 3 replications along measurement of ethylene production rate using ethylene meter obtained from a closed system method and represented by Michael-Menten equation. The temperature dependence was represented base on Arrhenius law. The model parameters ($K_{m_{C_2H_4}}$ dan $r_{C_2H_4 \max}$) are calculated using Lineweaver-Burk linier graph method. MM and MMC applications can be used to estimate ethylene production rate of Barangan banana. MM equation resulted that 11-week harvest age obtaining R² was higher than 10 weeks 0.95 while the 10-week harvested R² 0.94. MMC equation, the 11-week harvest age R² 0.95 was higher compared to 10 weeks R² 0.94. It could be predicted that MM and MMC equations can predicted the rate of ethylene production of Barangan banana

Key words: Barangan Banana, Respirations, Ethylene rate, Arrhenius, Michaelis-Menten Kinetic

1. Introduction

Ethylene has properties that can be detrimental because it can shorten the shelf life of horticultural products but on the other hand is beneficial because it can trigger and homogenize the ripening process (Genard M, 2005)

The Michaelis-Menten (MM) and Michaelis-Menten Cooperative (MMC) models are mathematical models used to estimate the rate of ethylene production based on enzymatic reactions. MM is a model that describes the reaction rate of ethylene based on substrate concentration (Copeland 2000). Binding of O₂ to improve the performance of active compounds (ACC oxide enzyme) in forming ethylene (De Wild *et al.* 1999). The activity of this enzyme will work optimally until the peak of the ethylene production and is directly proportional to the increase in O₂ levels. MMC equations, which indicates the performance of enzyme cooperativity in binding the substrate during the reaction is called the degree of cooperativity (h) (Sanders and De Wild 2003).



The MM and MMC models have been investigated to estimate the rate of ethylene production in fruits and vegetables (De Wild *et al* 1999; Sanders and De Wild. 2003; Mendoza *et al* 2016).. Diego *et al.* (2016) used the MMC model on estimating the rate of ethylene production of Cavendish bananas with 3 storage temperatures. The temperature of 15 °C produces R^2 0.98, 20 °C had a value R^2 0.97 and the temperature of 25 °C produces R^2 0.97. This model is also based on Michaelis kinetics-Menten. Mendoza *et al.* (2016) model ethylene in Cavendish bananas with the Michaelis-Menten enzymatic kinetics equation on a MAP (*Modified Atmosphere Packaging*) system with different temperatures, with the results of the MM model having a more predictive value than the distribution model. The MM model as a model for estimating the rate of ethylene production using enzymatic kinetics can only be used to predict ethylene production to the climacteric peak. (Diego *et al* 2016; Mendoza *et al.* 2016).

Estimation of ethylene production is very useful for carrying out proper postharvest handling both for inhibition and maturity regulation of bananas, especially Barangan banana, which is a local banana that has been designated as a superior fruit in the area of North Sumatra and has the potential as an export fruit. The aim of the study was to estimate the rate of ethylene production in Barangan bananas using the approach of the Michaelis-Menten enzymatic kinetics equation

2. Materials and Methods

2.1 Sample preparation

Barangan banana is obtained from PT. Perkebunan Nusantara VIII Parakansalak. Bananas used are harvested 10 and 11 weeks. After being washed with alum water (the concentration according to the SOP of PT PN VIII), Barangan bananas are packaged and on take it to the Agriculture and Food Product Engineering Laboratory of the Bogor Agriculture University. After arriving, It is re-sorted from mechanical damage. Bananas selected as the experiment sample were weighed and measured of volume using the Archimedes method. The volume of the material as a base determines the free volume in the *chamber*. Samples were prepared for the measurement of respiration and ethylene at three temperatures of 10, 20 and room temperature (27-29) °C with 3 replications.

2.2 Measurement of Respiration Rate (O_2)

Barangan respiration rate measurement uses a closed system (Hasbullah 2007). There are inserted into the chamber. The chamber is closed with a cover that has been equipped with two flexible plastic pipes as air intake and intake lines. The distance between the chamber and the lid is closed with plasticine to prevent air from entering the *chamber*. Data obtained in the measurement of respiration rate in the form of changes in O_2 gas concentration. The concentration of gas O_2 is measured using *Continuous Gas Analyzer (Shimadzu IRA-107)* with data collection intervals every 3 hours for 4 days due to peak respiration rate on day 4. Measurements are made at three different temperatures (temperatures of 10, 20, 28) °C. The rate of gas production O_2 (ml /kg-hour) during respiration in confined spaces is calculated by the equation of respiration (Fonseca 2002):

$$r_{O_2}(t) = \left(\frac{V}{w}\right) (y_{O_2 t} - y_{O_2 t+1})/\Delta t \quad (1)$$

Where r_{O_2} is consumption rate of O_2 each time (ml kg⁻¹h⁻¹), V is free volume (ml), w is sample weight (kg), y_{O_2} is O_2 mole fraction (it is the initial mole fraction, and t + 1 is the value of the mole fraction during the measurement) and Δt is the changing of time (hour)

2.3 Measuring Ethylene Production Rate

Along with the measurement of the concentration of O₂, the measurement of ethylene concentration in Barangan banana harvested (10 and 11) weeks at different temperatures (10, 20, 28) °C with ethylene meter (cosmos) for 4 days with 3 replications. The rate of ethylene production can be calculated by equations (Diego *et al.* 2016):

$$r_{C_2H_4} = \left(\frac{V}{W}\right) (y_{C_2H_4t} - y_{C_2H_4+1}) / \Delta t \quad (2)$$

Where $r_{C_2H_4}$ is ethylene production rate (ml kg⁻¹h⁻¹), V is free volume (ml), W is sample weight (kg), $y_{C_2H_4}$ is ethylene fraction mole, and Δt is the changing of time (hour)

2.4 Arrhenius

The rate of respiration and ethylene production is changed which according coefficient of to the storage temperature along respiration rate in enzymatic kinetics is the storage temperature (Wang *et al.* 2009). So that the effect of storage temperature on Michaelis-Menten kinetics formulation ($r_{C_2H_4}$) (4) and (5) stated in Arrhenius law (Diego *et al.* 2016):

$$k = k_0 e^{-E_a/RT} \quad (3)$$

where k is oxygen consumption rate (ml kg⁻¹h⁻¹), E_a is energy activation (kJ/mol), K_0 is constants (not depend on temperature), R is gas constants (8,341 J/mol), T is temperature of storage (°K)

2.5 Application of Michaelis-Menten Equations at Ethylene Production Rate

The equations of MM enzymatic kinetics (De wild *et al.* 1999) and MMC (Mendoza *et al.* 2016) in estimating the rate of ethylene production is based on the reaction between enzymes and O₂ of ethylene production with positive cooperativity (Sander and De Wild 2003).

$$r_{C_2H_4} = \frac{r_{C_2H_4} Y_{O_2}}{(K_{m_{C_2H_4}}) + Y_{O_2}} \quad (4)$$

$$r_{C_2H_4} = \frac{r_{C_2H_4 \max} (Y_{O_2})^h}{(K_{m_{C_2H_4}})^h + (Y_{O_2})^h} \quad (5)$$

Where $r_{C_2H_4}$ ethylene production rate (ml kg⁻¹h⁻¹), h degree of cooperation (Hill coefficient), $r_{C_2H_4 \max}$ the rate of ethylene production in the maximum state (parameter), $K_{m_{C_2H_4}}$ Michaelis-Menten constant Y_{O_2} O₂ mole fraction

Determination of value $r_{C_2H_4 \max}$ and $K_{m_{C_2H_4}}$ (4) and (5) using a Lineweaver-Burk linear curve (Coopeland, 2000). That will form the equation $y=ax+b$, value of $r_{C_2H_4 \max}$ calculated by the value of b , whereas $K_{m_{C_2H_4}}$ calculated by the value of a . Hill coefficient (h) (5) is calculated using equation (Mendoza *et al.* 2016):

$$\frac{1}{r_{C_2H_4}} = \frac{1}{r_{C_2H_4 \max}} (K_{m_{C_2H_4}} (Y_{O_2})^h + 1) \quad (6)$$

Equation 6 is a linear form of Equation (5) (Mendoza *et al.* 2016) making it easier to calculate Hill coefficient (h).

2.6 Model Validation

Validation in the Michaelis-Menten equation is done by calculating the coefficient of determinant (R^2) and the error in the equation used. R^2 is calculated by linear regression analysis (Abdullah *et al.* 2015) to explain the ability of independent variables in explaining the dependent variable and error was calculated by Sum

Square of Error analysis (Bhande *et al.* 2008). SSE (*Sum Square Error*) is one of the statistical methods to measure the difference the total of the true value of the value achieved by the model. Data validation uses replication of the data (1 data replication for validation and 2 data replications for model). SSE is calculated by equation (Pratama and Harjoko, 2015):

$$SSE = \sum_{i=1}^n (X_i - Y_i)^2 \quad (7)$$

Where X actual value , Y value achieved n data

3. Results and Discussion

3.1 Respiration rate

In this study, the measured respiration rate was consumption of O₂ Barangan bananas based on the temperature and time of harvest as show on in Fig 1. Harvesting 10 and 11 weeks which an increasing of temperature causes increase of the rate of consumption of O₂. This was due to the higher storage temperatures can lead to increased respiration rate of a horticultural products. A chemical reaction will take place faster by increasing the temperature, with the temperature being increased, the kinetic energy of the reacting molecules are increases, so that the reaction is faster than the low temperature (Genard M, 2005).

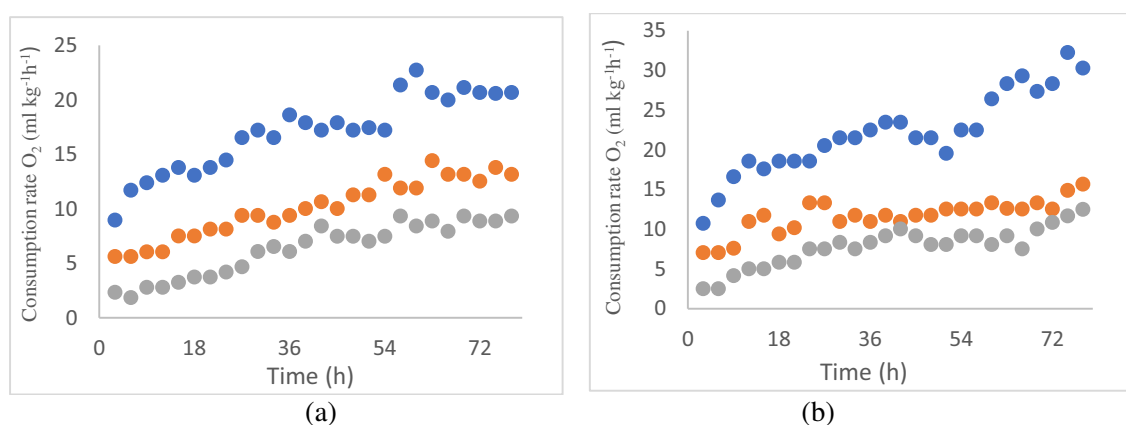


Figure 1. O₂ consumption rate of Barangan banana (a) week 10 (b) week 11 at temperature (10, 20, 28) °C

At the 10 weeks harvest, maximum rate of consumption of O₂ temperature (°C) 28,20,10 are 20.68, 13.16, 9.35 (ml k g⁻¹ h⁻¹), while the harvesting of 11 weeks, maximum consumption rate of O₂ is 30.30, 15.66, 12:52 ml k g⁻¹ h⁻¹). This result consistent with the research by De Wild *et al* (2003); Diego *et al.* (2016) in Cavendish bananas that the consumption of O₂ in bananas will increase when the storage temperature is increased, the rate of consumption of O₂ at low temperatures is smaller than the high temperature. Barry and Giovvanoni (2007) explains that the rate of consumption of O₂ is influenced by the temperature and age of harvesting, the higher storage temperature and the physiological of harvest of horticulture products, the consumption rate O₂ will be even greater.

3.2 Ethylene Production

Ethylene production of Barangan banana at 10 and 11 weeks at 3 different temperatures (10, 20, 28) °C show on as Figure 2.

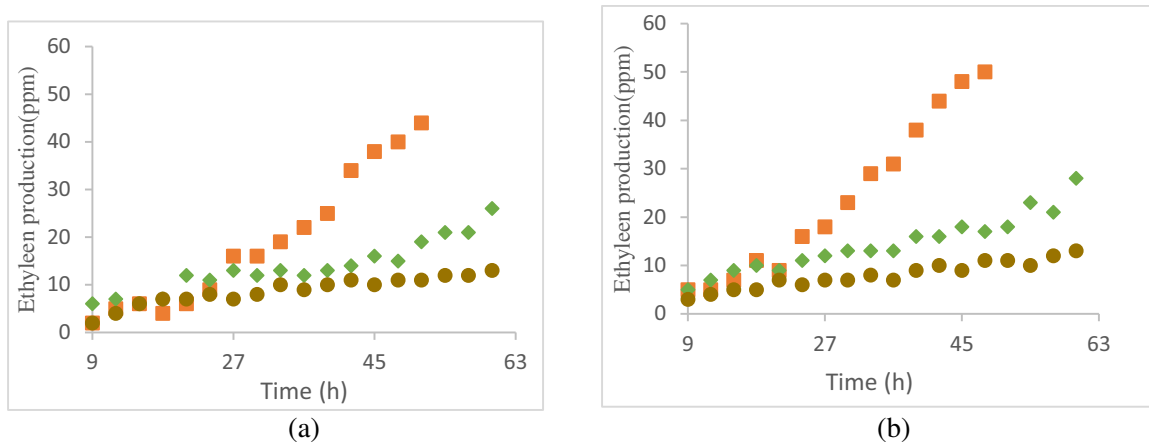


Figure 2. Ethylene production of Barangan banana (a) 10 week, (b) 11 week at temperature
 ■ 28 °C ◆ 20 °C, ● 10 °C

Production of ethylene measurement show that the ethylene production will increase during the increasing of temperature and due the physiological 11 weeks production the ethylene higher than 10 week. 10 weeks produce the ethylene maximum (13, 27, and 44) ppm while 11 weeks produce (16, 29 and 50) ppm as the maximum production. When the storage temperature going up, the metabolism of bananas will progress faster and ethylene produced will be high (Genard M 2005). This result consistent with study by Nurjannah (2009) where the ethylene production will increase with increasing storage temperatures and physiological age of horticulture product.

3.3 Arrhenius

The Arrhenius model application is preceded by the calculation of the value of k using Equation 5 to produce an equation calculating the value of k for each harvest age as show in Table 1.

Table 1. Activations energy using Arrhenius equations

weeks	Ea (kJ/mol)	k0	The equations of k
10 minggu	98.12	$2,66776 \times 10^{15}$	$k = 4.68902 \times 10^{15} e^{98.12/R.T}$
11 minggu	81.49	$2,43262 \times 10^{12}$	$k = 2,43262 \times 10^{12} e^{81.49/R.T}$

The equation of k (Table 1) is obtained from finding the k value for each temperature (10, 20, 28) °C so that the graph $1/T$ vs $\ln K$. will be used to find the E_a and k_0 values. There are entered into the Arrhenius equation (5) so that they get the k value equation in Table 1. The low of Activation Energy (E_a) indicate a product has a higher reaction speed (Khatir *et al* 2014). The results of this study were 10 weeks of harvesting have E_a 98.12 kJ/mol and 11 weeks of harvesting have E_a 81.49 kJ/mol so that the ethylene production speed of Barangan banana 11 weeks higher than 10 weeks as in Figure 2. Diego *et al.* (2016) explained that the lower E_a on the ethylene reaction will run faster so the ethylene production will be higher. Positive activated energy indicated that the effect of temperature has linier effect to ethylene production of Barangan banana (Diego *et ai.* 2016).

3.4 Estimate of Ethylene Production Rate of Barangan Banana

Estimating the rate of ethylene production at 3 different temperatures and two different harvest ages show on (Figures 3 and 4) based on the mole fraction using MMC and MM equations

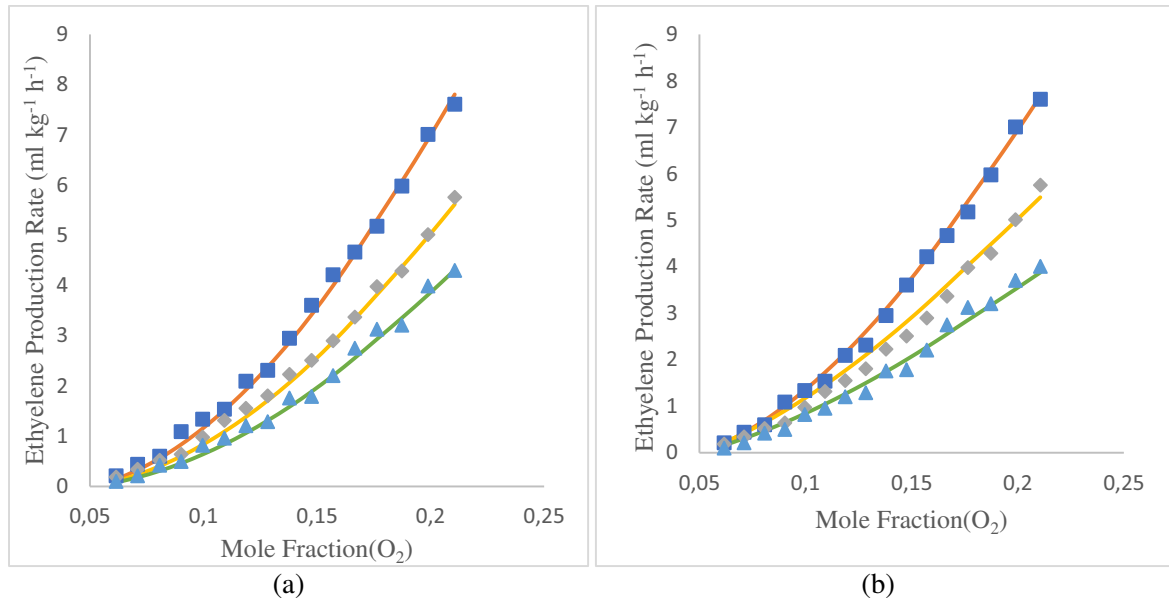


Figure 3. The result of measurement and result of ethylene production rate of Barangan banana using Michaelis-Menten Equations 10 weeks of harvested. a (MM),b (MMC) temperature (28, 20, 10) oC.
 ■ 28 oC, ♦ 20 oC, ▲ 10oC

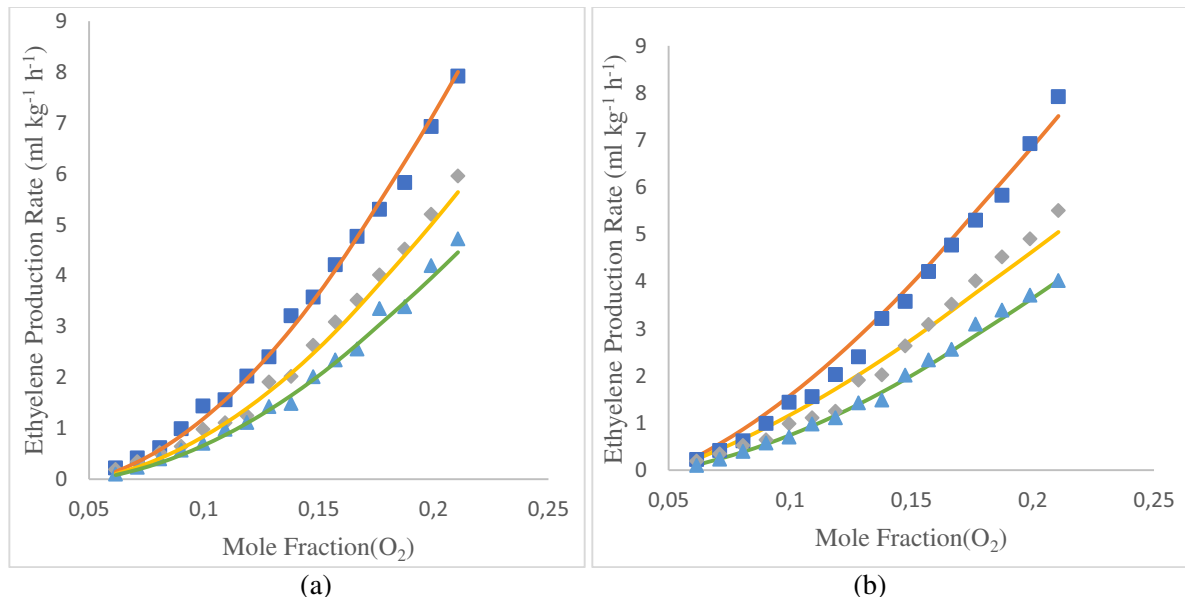


Figure 4. The result of measurement and result of ethylene production rate of Barangan banana Using Michaelis-Menten Equations 11 weeks of harvested. a (MM),b (MMC) temperature (28, 20, 10) oC.
 ■ 28 oC, ♦ 20 oC, ▲ 10 oC

The differences in the MM and MMC models can be seen in the model parameters produced as show in (Table 2).

Table 2. Parameter of equations MMC dan MM in estimated productions of ethylene rate

	10 weeks			11 weeks		
	28 °C	20 °C	10 °C	28 °C	20 °C	10 °C
MM						
$Km_{C_2H_4}$	$4.56 \pm 8.32 \times 10^{-3}$	$4.40 \pm 8.35 \times 10^{-3}$	$4.28 \pm 3.47 \times 10^{-3}$	$4.51 \pm 6.32 \times 10^{-3}$	$4.39 \pm 3.68 \times 10^{-3}$	$4.30 \pm 3.11 \times 10^{-3}$
$r_{C_2H_4 \max}$ (ml.kg/h)	$5.61 \pm 6.32 \times 10^{-3}$	$4.00 \pm 7.23 \times 10^{-3}$	$2.01 \pm 4.32 \times 10^{-3}$	$5.90 \pm 7.44 \times 10^{-3}$	$4.10 \pm 5.54 \times 10^{-3}$	$2.27 \pm 6.32 \times 10^{-3}$
R^2	0.94	0.93	0.93	0.95	0.95	0.92
SSE	1.15	1.11	1.19	0.97	1.11	1.23
MMC						
$Km_{C_2H_4}$	$4.56 \pm 8.32 \times 10^{-3}$	$4.40 \pm 8.35 \times 10^{-3}$	$4.28 \pm 3.47 \times 10^{-3}$	$4.51 \pm 6.32 \times 10^{-3}$	$4.39 \pm 3.68 \times 10^{-3}$	$4.30 \pm 3.11 \times 10^{-3}$
$r_{C_2H_4 \max}$ (ml.kg/h)	$5.61 \pm 6.32 \times 10^{-3}$	$4.00 \pm 7.23 \times 10^{-3}$	$2.01 \pm 4.32 \times 10^{-3}$	$5.90 \pm 7.44 \times 10^{-3}$	$4.10 \pm 5.54 \times 10^{-3}$	$2.27 \pm 6.32 \times 10^{-3}$
h	0.93	0.81	0.72	0.82	0.79	0.70
R^2	0.94	0.93	0.90	0.95	0.93	0.91
SSE	0.98	1.02	1.27	0.95	0.97	1.09

Based on model parameters produced, the harvest of 10 weeks and 11 weeks, the MMC and MM equation has a different R^2 . The MM model is more appropriate at the age of 11 weeks because it has a higher R^2 0.96. De Wild *et al* (1999) shortly explain that the differences in physiological age harvest will affect the results of the model (R^2) where the physiological harvest age is older, the value of R^2 produced will be better. Likewise with MMC models, harvesting age 11 weeks resulted in a value of R^2 is larger than the 10 weeks. This result is consistent with those reported by Sanders and De Wild (2003); (Diego *et al.* 2016) where the model MMC that older harvesting produce R^2 is larger

Based on Table 2, the value of the parameter h (coefficient hill) at 11 weeks harvest smaller than the harvest age of 10 weeks. The value of h illustrates the magnitude of the effect of O_2 during ethylene production. Sander and De Wild (2003) ; (Mendoza *et al.* 2016) describes the parameter values are affected by age physiological from the sample, the high of it make h value would be diminished. Copeland (2002) ; Castellanos (2016) explains that the rate of consumption of O_2 during high ethylene production, the value of h will be low. 11 weeks, the consumption rate of O_2 is greater than 10 weeks, so 11 weeks has a small value (h). The value of h for each temperature is different where the temperature of storage is high, the value of h will be higher.

The parameter values of Km every temperature is dissimilar. The km values in the MM and MMC equations describe the activities of enzyme in the ethylene process based on the level of consumption of O_2 (Segel 1993). Due the parameters as show in Table 2, the temperature is rising then the value $Km_{C_2H_4}$ at MM and MMC will be large, and low temperature the value of $Km_{C_2H_4}$ will be small. This result consistent with those reported by Sanders and De Wild (2003); Wang *et al* (2009) ; Mendoza *et al.* (2016) that parameter values $Km_{C_2H_4}$ both MM and MMC will decrease according to the decrease in storage temperature used. This is because the enzyme performance will experience inhibition at low temperatures and the performance of the enzyme have been effective at high temperatures (Copeland 2002). Consumption of O_2 at high temperatures will be greater so that the value $Km_{C_2H_4}$ both the MM and MMC equations will be even greater (Sanders and De Wild 2003)

The smallest SSE indicates the model is closer to direct measurement (Sari *et al.* 2014). As show in Table 2, SSE at 11 weeks smaller than the age of 10 weeks. This illustrates the MM and MMC equations can be used to predict the rate of ethylene production of Barangan banana. De wild *et al.* (2003), the physiological harvest age of bananas will influence the results of the models used where the harvest age is older, the SSE produced will be smaller.

The rate of ethylene production will increase along the increasing of temperature storage, the higher the storage temperature, can involve the rate of ethylene production. Rashid *et al.* (2012) explained that an increase of storage temperature is directly proportional to the increase in the rate of ethylene production where high temperatures will cause the enzyme working reaction (ACC Synthase) to increase, pushing the metabolic reaction in bananas will be going up, so the rate of ethylene production will increase.

4. Conclusion

The application of the MM and MMC equations can be used to estimating the rate of ethylene production of Barangan banana. The MM model, 11 weeks of harvest produced the highest R^2 value compared of 10 weeks harvesting with the value of R^2 0.96 while the age of 10 weeks the highest R^2 0.95. The MMC, harvest age 11 weeks produce the highest R^2 value of 0.94 at while 10 weeks harvested produces the highest R^2 0.94. The conditions of MM and MMC are more appropriate to use at 28 °C to predict the rate of ethylene production Barangan banana. Value $Km_{C_2H_4}$ on MM and MMC equations will going up while decreasing of storage temperature. MMC equation is more appropriately used at 11 weeks harvested where the value of h on 11 weeks low than 10 weeks of harvested.

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