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OPTIMISATION OF ETHYLENE TREATMENT CONDITIONS FOR OIL PALM FRUIT DETACHMENT USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Ethylene gas can be applied to oil palm fresh fruit bunches (FFB) to enhance fruit detachment and produce fresh loose fruits (LF) that could be processed separately to reduce oil loss due to empty fruit bunch (EFB) absorption during the milling process. Response surface methodology (RSM) with central composite design (CCD) was employed to achieve optimum fruit detachment in oil palm FFB as the main response with two experimental variables, which were ethylene concentration (500 to 2000 ppm) and incubation period (18 to 24 hr). The proposed model showed an R^2 *value of 0.85 where the experimental variables were significant to the result. From the model, 26% of the targeted fruit detachment was attained with ethylene concentration of 1102.7 ppm and an incubation period of 21.5 hr. The application of optimum ethylene gas and incubation time from the study can be then tested on a pilot scale to reduce optimisation time.*

Keywords: ethylene, fresh fruit bunch, fruit detachment, loose fruits, RSM.

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INTRODUCTION

Oil palm is the main commodity crop in Malaysia and has contributed significantly to the country's economic development. In 2020, the Department of Statistics Malaysia reported that palm oil accounted for about 38% of Malaysia's agricultural output value and contributed to 3% of its gross domestic product in 2019 (Department of Statistics, 2020).

One of the most important attributes in determining the crude palm oil (CPO) quality is the harvested fresh fruit bunches (FFB). Typically, FFB quality is classified based on colour, shape, and ripeness (Basiron, 2007). Harvesting FFB at the right ripeness will potentially maximise oil yield as individual fruit can further accumulate oil and achieve the highest oil content before detachment from the bunch (Mohanaraj and Donough, 2016). Harvested FFB will then be delivered to an oil mill for a series of milling processes to produce CPO. The mass ratio between extracted CPO and processed FFB is normally used to estimate oil extraction rate (OER) (Corley and Tinker, 2003) and serves as an important key performance indicator for palm oil mills (Zulkefli *et al.,* 2017). Aside from FFB quality, many other factors could impact OER such as oil content in oil palm fruitlets, palm age, soil conditions, climate, harvesting practices and oil extraction efficiency in mills (Chew *et al*., 2021).

Nadzim *et al.* (2020) reported the OER in our country has fluctuated for the last 20 years. Low OER implies low oil productivity, low revenue, and some oil loss during a particular processing stage (Chang *et al.*, 2003; Chew *et al.*, 2021; Zulkefli *et al.*, 2017). There are several sources of oil loss in the conventional palm oil milling process including empty fruit bunches (EFB) and unstripped bunches (USB) after threshing, press cake fibre from screw press, steriliser condensate and sludge from the separator. The approximate oil loss encountered in EFB ranges from 0.3% to 0.5% with a ratio to FFB (Nadzim *et al.*, 2020; Ng, 1994). To minimise oil loss caused by EFB absorption during sterilisation, we explored fruit detachment induced by ethylene gas in FFB so that only high-quality loose fruits will be processed in mills. Our previous study by Balakrishnan *et al.* (2021) showed that fruit

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detachment achieved was 29.4 ± 1.9% incubated with 750 ppm ethylene gas for 24 hr. The ethylene gas and incubation period were only tested at 500 ppm to 1250 ppm for 24 hr. A similar study by Nualwijit and Lerslerwong (2014) used higher ethylene concentration with the same incubation period applied on the unripe FFB stimulated fruit abscission. There is no prior published research on the optimum conditions of these two parameters: ethylene concentration and incubation period on FFB fruit detachment other than from our previous study (Balakrishnan *et al.*, 2021; Chew *et al.*, 2021). From our findings, both ethylene concentration and incubation duration have more influence on fruit detachment. Therefore, we decided to deploy the Response Surface Methodology (RSM) to further investigate the combined effect of these two parameters at the lab and pre-pilot scale.

RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effect of variables, and searching for optimum conditions of variables for desirable responses (Montgomery, 2017). RSM explores the relationships between several experimental variables and one or more responses could be employed (Bhagwat *et al.*, 2021). Consequently, the objective of this article was to determine the processing parameters and functional relationship of exogenous ethylene concentration and incubation period to achieve optimum fruit detachment with the RSM tool.

MATERIALS AND METHODS

Plant Material

Oil palm FFBs of the *Tenera* variety were harvested from the commercial fields in Banting and Carey Island, Malaysia. Only ripe bunches that had undergone a colour change (from deep violet to yellowish-orange), according to the ripeness standard established by MPOB (2003) (*Table 1*) and weighing between 14 to 20 kg were selected for this study. The bunches were transported to the research facility within 2 hr of harvesting.

TABLE 1. OIL PALM FRUITS RIPENESS STANDARD

Fruit ripeness	Description
Unripe	No fruitlets detached from a fruit bunch
Under-ripe	1 to 10 fruitlets detached from a fruit bunch
Ripe	>10 fruitlets detached and 50% of fruitlets are still intact in a fruit bunch
Over-ripe	> 50% of the fruitlets detached from a fruit bunch

Source: MPOB (2003).

Response Surface Methodology Experimental Design

Central composite design (CCD) of RSM was used to determine the number of experiments to be evaluated for optimisation of variables and response using State-Ease software (Design Expert v11.1.2.0). This CCD consisted of two independent variables, which were ethylene concentration and incubation period to investigate the optimum condition that gives a better percentage of fruit detachment, known as the response in this experiment (*Table 2*). The minimum, intermediate and maximum values of each variable were coded as -1, 0 and +1, respectively. The experimental design consisted of 11 experimental runs, including three centre points, four factorial and four axial points to cover the possible combinations of variables and their effect on the investigated response. Random experimental orders were performed in triplicate to minimise the unexpected variability effects on the observed response due to systematic error.

TABLE 2. LEVELS AND VALUES OF THE INDEPENDENT VARIABLES FOR THE CENTRAL COMPOSITE DESIGN OF RESPONSE SURFACE METHODOLOGY

Experiment variables	Coded factors	Levels of coded factor		
		-1	0	1
Ethylene concentration, x_1 (ppm)	A	500	1 250	2 000
Incubation period, x_2 (hr)	B	18	21	24

Experiment Set-up

Ethylene treatment was performed on individual ripe bunches and each treatment consisted of five replicates (n=5). The bunches were individually placed inside a covered incubation box (V=150 L). Ethylene gas (Gaslink, Malaysia) was transferred into the box by a fixed pressure at 0.3 bar, with a flowrate of 0.1 L min^{-1} at the designed concentration and incubation period as presented in *Table 2*. The gas concentration was monitored using a portable gas detector (PG610, Henan Inte Electrical Equipment Co., Ltd).

For the validation study, optimum points determined by the RSM model including ethylene concentration of 1102.73 ppm at 21.55 hr were used at the lab and pre-pilot trial. Approximately 150 kg of ripe FFB were collected and placed inside a larger covered incubation bin (V=1100 L) for validation study during the pre-pilot trial. Five replicates (n=5) of experimental runs were performed for both control and treatment. Post ethylene treatment, the fruitlets on FFB were hand stripped manually and all the detached fruits were weighed.

Fruit detachment was determined according to the Equation (1):

$$\text{Fruit detachment } (\%) = \begin{pmatrix}\text{Total weight of} \\ \text{detached fruits / total} \\ \text{weight of bunches}\end{pmatrix} \times 100\% \quad (1)$$

Statistical Analysis

Design Expert v11.1.2.0 was used for statistical and graphical analyses of the experimental data. Statistical significance was evaluated using Fisher's F-test. The quality of the predictive model was statistically evaluated by using the coefficient of determination. All experiments were carried out in triplicate and the average values were reported.

RESULTS AND DISCUSSION

Experimental Design Matrix and Results Analysis

The design matrix to optimise fruit detachment is summarised in *Table 3*. The correlation between two variables and response was developed by CCD. The fruit detachment obtained from experiment data ranged from 11.1% to 30.8%. Three replication of the central point (standard order 9 to 11) were used to predict the error that represents the accuracy of the experiment. The obtained standard error from the actual experiment was 1.5, which was higher than the optimum range of lesser than 1.0 (Feng *et al*., 2017). The high standard error may be due to varied weight and ripeness conditions of different biological replicates of FFB randomly selected from the commercial field for this experiment.

Based on the results, fruit detachment increased with incubation time at a fixed ethylene concentration. At 500 ppm of ethylene concentration, detachment achieved was only at 11.1% but increased to 23.7% and 27.8% after 21 hr and 24 hr of incubation. The observation can be due to the autocatalytic reaction of ethylene response in the FFB that could have started only after 18 hr with exposure of lower exogenous ethylene initially (500 ppm) as displayed by other climacteric fruits such as tomato, papaya, and banana (Fabi and do Prado, 2019; Liu *et al*., 1999; Yokotani *et al*., 2009). Autocatalysis of ethylene production is a characteristic feature of the ripening of climacteric fruits in which a massive increase in ethylene production is triggered by exposure to ethylene at a concentration above a threshold level (Liu *et al*., 2015). In this study, endogenous ethylene produced by FFB was insufficient, so when exogenous ethylene was applied, it induced an autocatalytic ethylene production system in FFB.

The results showed that with similar exposure time at higher exogenous ethylene of 1250 ppm and 2000 ppm, there was a drastic increase in fruit detachment, which indicated that a large amount of ethylene was sufficient to induce an autocatalytic reaction from basal level (system 1, S1) to ripening and senescence-associated system 2 (S2) (Hewitt and Dhingra, 2020; Yokotani *et al*., 2009). Under the S1 mode in non-climacteric fruits such as sweet cherry, kiwi, and pineapple, ethylene biosynthesis terminates upon reaching the basal level and is not affected by the ripening process (Bapat *et al*., 2010; Hewitt *et al*., 2021). In contrast, the ethylene biosynthesis in climacteric fruits such as oil palm, apple, banana, and mango increase during the transition from preclimacteric

TABLE 3. VARIABLES, LEVELS, AND THE RESPONSE OF FRUIT DETACHMENT BASED ON ETHYLENE CONCENTRATION AND INCUBATION PERIOD

Std. order	Variables levels[a]		Response		Residual
	Ethylene concentration, *A* (ppm)	Incubation period, *B* (hr)	Fruit detachment (%)	Predicted value (%)	
1	500 (-1)	18 (-1)	11.10	12.96	-1.86
2	2 000 (1)	18 (-1)	22.50	22.98	-0.48
3	500 (-1)	24 (1)	27.80	28.78	-0.98
4	2 000 (1)	24 (1)	25.70	25.29	0.41
5	500 (-1)	21 (0)	23.70	20.87	2.83
6	2 000 (1)	21 (0)	24.20	24.13	0.07
7	1 250 (0)	18 (-1)	23.50	20.95	2.55
8	1 250 (0)	24 (1)	30.80	30.01	0.79
9	1 250 (0)	21 (0)	22.40	25.28	-3.08
10	1 250 (0)	21 (0)	27.40	25.48	1.92
11	1 250 (0)	21 (0)	23.30	25.48	-2.18

Note: [a] Coded level is with brackets.

(S1) mode to climacteric (S2) mode (Chen *et al.*, 2018) upon induction by exogenous ethylene (Gwanpua *et al.*, 2018). Therefore, with higher concentrations of ethylene at 1250 ppm and 2000 ppm, it was observed that 23.5% and 22.5% of detachment were achieved at 18 hr of incubation, which indicated that autocatalytic reaction of ethylene can be activated much earlier by increasing ethylene concentration. The incubation time variable was not set lower than 18 hr taking into consideration the duration from post-harvest FFB and typical daily mill operation.

Central Composite Design Model Development and Analysis

The sequential model sum of squares for this study was shown in *Table 4*. The highest-order polynomial was used to select the appropriate model. Two-factor interaction (2FI) model was proposed with *p*-value of 0.0578 ($p<0.1$) and high F-value of 5.13.

To assess the fitness and reliability of the proposed model, three main tests were used, namely, significant of terms, lack-of-fit and regression model. An examination of the significance and fitness of the 2FI model based on the *p*-value and F-test was carried out to ensure the sufficiency of the suggested model (*Table 5*). Based on the ANOVA model, the *p*-value was 0.0128, indicating that the model was statistically significant.

The model is reliable and reproducible based on the regression coefficient (R^2=0.85), coefficient of variation (C.V.%=10.53%) and precision residual sum of square (PRESS=140.62), respectively. In this study, the PRESS is greater than 4, indicating there is a good correlation between experimental data and the predicted model. The incubation period was found to be the most influential factor. The non-significant lack of fit (*p*-value=0.6093) confirms the significance and adequacy of the model.

RSM Model Validation

Based on the obtained results, the model built for fruit detachment using ethylene concentration was in Equation (2). This equation was generated from the coded levels, as shown in *Table 2*. The coded equation is useful for identifying the relative impact of the variables by comparing them with the factor coefficients:

$$\text{Fruit detachment (\%)} = 25.48 + 1.63A + 4.53B - 3.38AB - 2.98A^2 \quad (2)$$

The equation in terms of the actual factors:

$$\text{Fruit detachment (\%)} = -56.63 + 0.05x_1 + 3.39x_2 - 0.002x_1x_2 - 5.30\times10^{-6}x_1^2 \quad (3)$$

A graphical representation of the model's quality was illustrated in *Figure 1a*. Predicted data by ANOVA *vs.* actual values for fruit detachment showed that the model almost formed a straight line. The residuals plot of the actual value (*Figure 1b*) showed that all the points were located within the acceptable range and were close to the centre line within the threshold cut-off at ± 4.8819. This indicated that the experimental data was adequately fitted to the model and can be employed to assess, evaluate, and predict the interactions between the significant variables in obtaining high fruit detachment efficiency.

Parameter Optimisation Based on RSM

The three-dimensional (3D) response surface plot (*Figure 2*) was facilitated to study the effects of variables and their interaction on FFB fruit detachment based on the developed model (Equation 2).

The 3D plot illustrated the fruit detachment increased with increasing ethylene concentration and incubation period. It was found that the optimum ethylene concentration and incubation period were 1102.73 ppm and 21.55 hr, to achieve targeted fruit detachment set at 26%. Based on our previous work, the FFB outer layer consists of 25%-28% of the total bunch weight where the fruits can be easily detached compared to the middle and inner layers (Balakrishan *et al.*, 2021). This was confirmed when 26% of fruit detachment was achieved from our preliminary trials.

TABLE 4. SEQUENTIAL MODEL SUM OF SQUARES

Source	Sum of squares	Degree of freedom	Mean square	F-value	*p*-value (prob>F)	Remark
Mean *vs.* total	6 259.43	1	6 259.43			
Linear *vs.* mean	139.31	2	69.66	5.18	0.0361	
2FI *vs.* linear	45.56	1	45.56	5.13	0.0578	Suggested
Quadratic *vs.* 2FI	24.30	2	12.15	1.61	0.2892	
Cubic *vs.* quadratic	8.08	2	4.04	0.4077	0.6972	Aliased
Residual	29.73	3	9.91			
Total	6 506.42	11	591.49			

TABLE 5. ANOVA RESULTS FOR RESPONSE SURFACE MODEL

Source	Sum of square	DF	Mean square	F-value	*p*-value (prob>F)	Remark
Model	209.10	4	52.27	8.28	0.0128	Significant
A, ethylene concentration	16.01	1	16.01	2.53	0.1625	Not significant
B, incubation period	123.31	1	123.31	19.52	0.0045	Significant
AB	45.56	1	45.56	7.21	0.0362	Significant
A^2	24.22	1	24.22	3.83	0.0979	Significant
Residual	37.89	6	6.32			
Lack of fit	23.69	4	5.92	0.8336	0.6093	Not significant
Pure error	14.21	2	7.10			
Corrected total	246.99	10				
Standard deviation	2.51		R^2	0.8466		
Mean	23.85		Adjusted R^2	0.7443		
C.V.%	10.53		Predicted R^2	0.4307		
Press	140.62		Adeq. precision	10.0662		

Note: Significative at a significance level of 0.1.

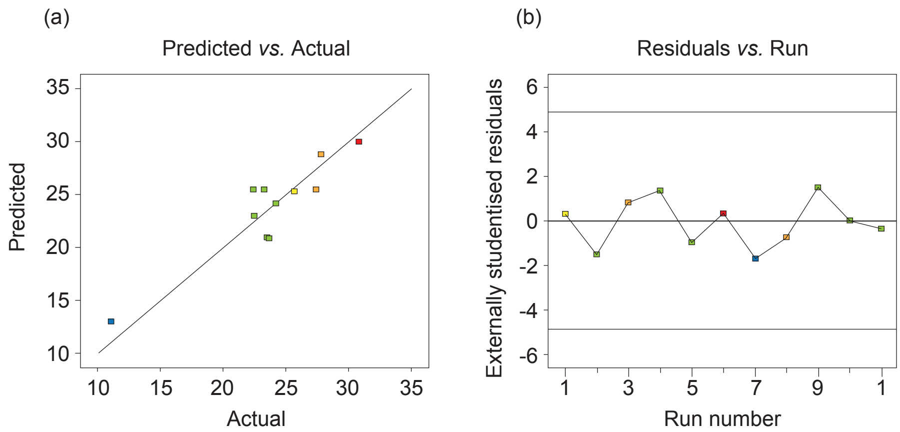
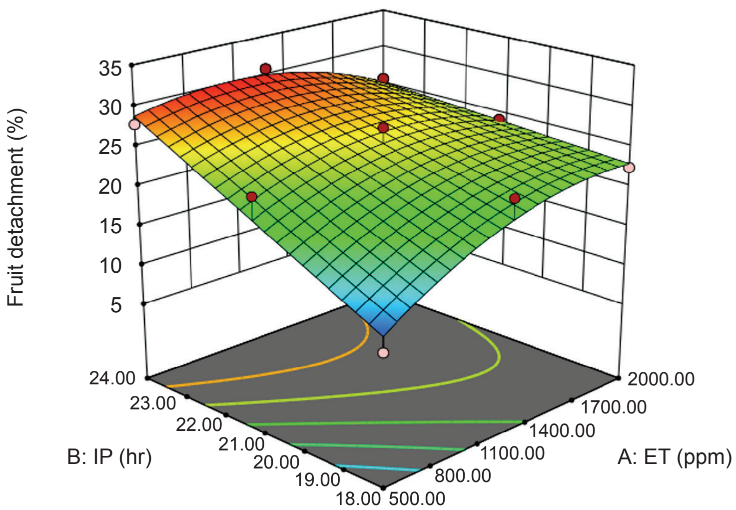


Figure 1. Diagnostic plot for developed model adequacy. (a) Actual response vs. *predicted response for fruit detachment, (b) Studentised residuals* vs. *run number, the threshold cut-off at ± 4.8819.*



Note: ET - ethylene concentration; IP - incubation period.

Figure 2. Three-dimensional response surface design of the fruit detachment vs. *ethylene concentration and incubation period.*

TABLE 6. COMPARISON BETWEEN ACTUAL AND PREDICTED VALUE OF FRUIT DETACHMENT AT THE OPTIMUM POINTS

Validation	Optimum point		Predicted fruit detachment (%)	Actual fruit detachment (%)
	Ethylene concentration (ppm)	Incubation period (hr)		
Lab	1 102.73	21.55	26	27.94 ± 1.38
Pre-pilot	1 102.73	21.55	26	27.00 ± 1.12

Model Validation

Model validation was performed using the optimum point of variables determined in this study (*Table 6*) for both lab and pre-pilot trials. Based on the model, fruit detachment was set at 26%, while the lab and pre-pilot give the average actual fruit detachment of 27.94 ± 1.38% and 27.00 ± 1.12% with insignificant difference (p=0.117 and p=0.197), respectively. Therefore, the results revealed that predicted responses from RSM were similar to experimental responses which were displayed in experimental trials. This demonstrated that the RSM is a good tool in ethylene concentration and incubation period optimisation for fruit detachment.

CONCLUSION

The fruit detachment study with multiple variables was successfully deployed using RSM through designing, analysing and discovering the optimum point, as well as evaluating the effects of variables. A 2FI model with R^2 value of 0.85, was effectively applied to explain and predict the response of fruit detachment. Numerical optimisation for targeted fruit detachment of 26%, was predicted to be achieved at ethylene concentration of 1102.73 ppm and an incubation period of 21.55 hr. The model was validated and found to have similar responses between predicted and actual results in both lab and pre-pilot trials. The application of ethylene is a realistic and novel solution for the palm oil industry that may revamp the oil palm mill process to reduce the oil losses through separate processing of high-quality loose fruits. However, the application of this technology in a commercial setting must be tested using the model's optimised variables as a proof of concept.

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