Abstract. The present waste management practices have adverse environmental impacts at the same time costly. Approximately, 80% of the Malaysian municipal wastes including organic wastes are usual disposed into landfills. Hence, transformation organic wastes not only providing economic and environmental benefits but has given waste a value. This study focused on synthesis of biodiesel from *H. illucens* pre-pupae fed with fruit waste and food waste. The objective was to evaluate interactions between the variables including catalyst loading, reaction time versus fatty acid methyl esters (FAME) yield (wt %), temperature and methanol to sample mass ratio. The response surface methodology (RSM) was used to investigate the bioconversion optimization process. Optimal biodiesel yield based on fruit waste achieved was 96.15 % at 51°C; 8.3:1 methanol: mass ratio; 253 min and 15.1 % catalyst. Furthermore, the optimal yield obtained from the second set of optimization using lipids of pre-pupae derived from food waste was achieved at 94.63 %. The optimum conditions for reaction temperature was 71°C, with methanol to mass ratio of 6.8:1, at reaction time of 254 min and catalyst loading of 7.0 v/v%. The properties of FAME produced were in accordance with EN 14214 and ASTM 6751 biodiesel standards.

Keywords: Biodiesel, Organic waste, *H. illucens* larvae, Fatty acid methyl ester (FAME).
1. Introduction

Minimising and managing all waste for better application of waste is one of the sustainable goal developments (SDG). The targets focused on environmentally sound management of all waste through prevention, reduction, recycling and reuse (targets 12.4 and 12.5) and reduction of food waste (target 12.3) [1]. The production of biofuel from valorisation of organic waste has been put forward as the key factor for resolving our dependency over the use of non-renewable sources and energy issues [2-3].

For instance, utilisation of insect’s larval of H. illucens for transformation of organic waste to valuable product such as biodiesel and alternative feed has been intensively studied [4-9]. Embedding H. illucens in waste transformation processes prominently reduce ecological impact affected by organic waste disposal. In addition, the conceptual implementation based on waste transformations via insect delivers various benefits such as reduction in waste and greenhouse gas (GHG) emissions, cost savings, nutrient conversion by upcycling of waste and providing passive income to lower middle income groups by selling insect protein and biofertiliser derived from waste conversion [10]. Moreover, this concept could be a framework for circular economy whereby waste generated from the industries are being minimised and converted into valuable resources rather than being discarded directly [2, 11].

Studies have demonstrated that H. illucens lipids could serve as a nonconventional biodiesel feedstock and high content of lauric acid could also serve as antimicrobial agents [12-13]. Insects use for biodiesel offers more sustainable solution than that of edible and non-edible plant-based oil, oleaginous microorganism and animal waste. This can be explicated by the easy availability and fast growing insect, able to valorise huge amount of various perishable waste, it is a non-commodity, it does not have a negative impact on water and soils, as well as not competing for land used by food crops and it does not require large land space unlike Jatropha. Moreover, it is a non-pest insect and not a disease carrier [14-15].

One should take note that, selection of insect feeding regime is crucial as it could affect the larval nutritional content and its proliferation. Lipids are one of the major components accumulated in the larvae biomass during their metabolism of nutrient; the process which known as bioconversion. The accumulation of lipids differs at different the larval stages. Hence, the degree of fatty acids saturation will greatly affect the biodiesel properties.

This continuance study focused on the synthesis of biodiesel using prepupae of H. illucens derived from fruit waste and food waste. The ultimate objective was to investigate and gain an in-depth understanding on the reaction interaction between the variables (temperature, methanol to sample mass ratio, catalyst loading and reaction time versus FAME yield (wt %). In order to achieve the optimal transesterification condition, a statistical experimental design and RSM analysis were used to facilitate the study findings.

2. Materials and Methods

Feedstocks of H. illucens per-pupae were originated from the continuation of our previous study [4]. Production of fatty acid methyl ester (FAME) were carried out using two different sets of larvae which fed on fruit waste (FrW) and food waste (FW), until it became prepupae. Thereafter, the prepupae were harvested, cleaned and further dried in an oven at 105°C for 24 hours. The dried prepupae were stored in a container for further use.

2.1. In-situ Transesterification of FAME

The experimental design according to Box-Behnken consisting 30 experiments where-by each experiment was done in triplicates to conduct in-situ transesterification reaction via ultra-sonication. The reaction was conducted in a series of parameters based on Box Behnken design. Ultrasonic frequency at 60 Hz was used throughout the in-situ transesterification reaction. FAME produced was further separated to remove crude glycerol. Purification of FAME was carried out by washing with warm distilled water (70°C). Washing procedure was repeated until no turbidity was observed [4]. Prior solvent evaporation, anhydrous sodium sulphate was added into the FAME to absorb the moisture content. Sample bottles were used to store the collected FAME sample for further analysis. The yield of FAME was determined using Eq. (1) [6].

\[
\text{Yield} = \frac{\text{weight of FAME}}{\text{weight of oil}} \times 100\%
\] (1)

2.2. Analysis and Characterization of FAME

The optimized yields of FAME were analyzed using gas chromatography with mass spectrometer (Shimadzu GCMS QP2010) and capillary column packed with 70% of cyanopropyl polysilphenylene-siloxane (60 m × 0.32 mm i.d; 0.25 μm film thicknesses). The GC operating condition was set in accordance to our previous study [4]. The profiling of FAME was compared between the relative retention times of 37 component FAME mixtures (Sigma Chemical Co., St Louis, MO, USA). The optimized yield of FAME samples was further characterized for biodiesel properties according to ASTM D6584.

2.3. Experimental Design

To study the response pattern and to determine the optimum combination of four variables at three levels in the reaction process, the Box–Behnken experimental design was used. As shown in Table 1, these variables
were coded as A, B, C and D for reaction time (min), catalyst loading (%), ratio of methanol to sample mass and reaction temperature (°C) respectively. In order to achieve the experimental response of FAME yield, a total of 30 experiments were conducted individually.

2.4. Statistical Analysis

A statistical software package “stat ease design expert” version 8.0 was used to determine the graphical analysis and regression data obtained from the experimental plan. For the design experiment for transesterification, the maximum value of FAME yield was considered as the response of process. Using the response surface regression via the polynomial Eq. (2), the obtained experimental data was subsequently analyzed and investigated.

\[
Y = \beta_0 + \sum_{j=1}^{4} \beta_j X_j + \sum_{j=1}^{4} \beta_{jj} X_j^2 + \sum_{j=1}^{4} \sum_{j=2}^{4} \beta_{jj} X_j X_j + \varepsilon
\]

where \(Y\) is the response, \(i\) and \(j\) are the linear and quadratic coefficients respectively, \(X_i\) and \(X_j\) are the uncoded independent variables, \(k\) is the number of factors studied and optimized in the experiment. Equation was also validated by carrying out confirmatory experiments. Based on the value of correlation (\(R^2\)), the quality of the established model was determined. Also, the statistical significance of the model was performed by analysis of variance (ANOVA) and indicated by the values of regression and mean square of residual error.

3. Results and Discussion

The potential use of \(H.\) illucens pre-pupae as biodiesel feedstock was streamlined to two batches. The first batch of pre-pupae was fed with fruit waste and the second batch was fed with food waste. The pre-pupae derived from these feeds were selected for its better growth performance and highest lipid accumulation. Prepupae biomass derived from these feeds were used for optimization of yield of FAME.

In-situ transesterification of via ultra-sonication method was employed in this research. The combinations of reaction variables were conducted based on the Box Behnken experimental design which consists of 30 runs (Table 1). The Box Behnken respond surface was used to evaluate the interactions of the reaction variables and subsequently used to optimize the respond. Finally, the optimized yield of FAME was assessed for its potential properties as alternative fuel source.

Furthermore, prior optimizations, preliminary for fruit waste and food waste study were carried out. These preliminary studies were conducted to determine the suitable range for the optimization of FAME yield. The initial results for fruit wastes showed that, at lowest temperature (50°C) and lowest methanol to mass ratio of 6:1, the yield of FAME was found to be highest. The yield of FAME increased linearly with increasing of catalyst loading and reaction time. However, the yield of FAME decreased with further increased in reaction temperature (above 50°C) and methanol to mass ratio (above 6:1).

In addition, the initial results for food wastes showed that, the yield of FAME increases with reaction temperature from 50°C and remained almost plateau at 80°C. As observed, the yield of FAME increases with the increased of catalyst loading and reaction time. However, the yield remained constant starting at catalyst loading of 10 wt% and reaction time of 200 mins, respectively. The range of methanol to sample ratio between 6:1 and 9:1 displayed the highest yield of FAME. Therefore, based on the preliminary results obtained from fruit waste and food waste, the ranges of the variables were able to be determined.

3.1. The Statistical Analysis of Variables

Experimental and predicted FAME yield for FrW and FW obtained from the experiments showed the lowest yield at a reaction temperature of 45 and 65°C, respectively. The experimental yields for FrW obtained were between 44-61 % and the predicted yields obtained were between 49-62 %. At 50°C and 255 min, maximum FAME yield obtained were 96 and 94 % based on experimental study and prediction, respectively. The optimum reactants required were mass ratio of 6:1 and 20 v/v% of catalyst.

For FW, the experimental yields obtained were between 51.92-92.53 %. Meanwhile, the predicted yields obtained from the RSM output were between 47.44-90.48 %. Overall, the set of experimental yield obtained was close to the predicted yields as determined from the RSM output. The tolerance of the yields obtained was approximately ± 5 % when comparing with the predicted yield.

Figures 1 and 2 illustrate the correlation between the experimental and predicted yield of FAME derived from pre-pupae fed with fruit waste and food waste, respectively. The correlation coefficient (\(R^2\)) achieve for both were \(R^2 >0.80\) indicating a good agreement between the predicted and experimental yield of biodiesel in these regression models.
Fig. 1. Correlation between the experimental and predicted yield of FAME derived from pre-pupae fed with fruit waste.

Fig. 2. Correlation between the experimental and predicted yield of FAME derived from pre-pupae fed with food waste.

The yield of FAME derives from dried pre-pupae of H. illucens fed with fruit waste and food wastes were tabulated in Table 1. The yields of FAME obtained were studied on the interaction between four variables (temperature (A), methanol to sample mass ratio (B), reaction time (C) and catalyst loading (D)). Table 1 shows the experimental and predicted yields of FAME obtained from each of the reaction condition.

Table 2 summarized the statistical analysis and lack of fit test. The result is found to be appropriately fit into a quadratic term of coded units. The results obtained showed the summary of statistic and lack of fit testing, evaluating on regression equation and determination coefficient (R^2). The standard deviation (SD) achieved for FrW at 3.83 was the lowest amongst the rest of the

Table 1. Results of the experimental design for FAME derived from dried pre-pupae of H. illucens fed with fruit waste and food waste.

| Fruit waste (FrW) | Food waste (FW) |
|-------------------|------------------|
| Variable          | FAME yield (%)   | Variable          | FAME yield (%)   |
| A  B  C  D       | Experimental     | A  B  C  D       | Experimental     |
| 45 6 240 20      | 55               | 65 3 255 6       | 60               |
| 45 3 255 20      | 44               | 65 9 255 6       | 54               |
| 45 6 255 15      | 61               | 65 6 255 9       | 68               |
| 45 6 270 20      | 58               | 65 6 255 3       | 51               |
| 45 6 255 25      | 57               | 65 6 270 6       | 63               |
| 45 9 255 20      | 53               | 65 6 240 6       | 56               |
| 50 6 255 20      | 90               | 65 9 255 6       | 60               |
| 50 3 240 20      | 64               | 70 6 255 6       | 90               |
| 50 6 255 20      | 95               | 70 6 240 9       | 80               |
| 50 6 270 25      | 91               | 70 6 240 3       | 64               |
| 50 6 240 25      | 55               | 70 6 270 9       | 81               |
| 50 9 255 15      | 94               | 70 6 270 3       | 60               |
| 50 3 255 15      | 65               | 70 9 270 6       | 71               |
| 50 3 255 25      | 78               | 70 6 255 6       | 93               |
| 50 9 270 20      | 83               | 70 9 240 6       | 83               |
| 50 6 240 15      | 80               | 70 3 240 6       | 66               |
| 50 6 255 20      | 94               | 70 3 270 6       | 68               |
| 50 9 240 20      | 70               | 70 6 255 6       | 92               |
| 50 6 255 20      | 95               | 70 3 255 9       | 74               |
| 50 6 270 15      | 79               | 70 9 255 9       | 79               |
| 50 6 255 20      | 93               | 70 6 255 6       | 86               |
response surface terms. Low standard deviation value or near to zero indicate good interaction between the respond variables. This inferred that, the variation or the dispersion of the data point obtained in Fig. 1 and 2 were tending to be close to the mean.

For FrW, the value of the determined ($R^2$) achieved was 0.9687 indicating that the sample variation of 96.87% for FAME yield was attributed to the independent variables and only 3.13% of the total variations were not explicated by the respond surface interaction (Table 1). Similarly, the $R^2$ achieved for FW was 0.977. Moreover, the value for adjusted determination co-efficient for FrW (Adj. $R^2$ = 0.9374) and FW (Adj. $R^2$ = 0.9523) showed comparably high, which also signified excellent interaction between the respond surface terms. On the basis of the results obtained, a high $R^2$ (> 0.8) justifies that there is good correlation between the model and experimental data.

The coded term of Eq. (2) represents the reaction yield for a particular set of reaction conditions. Each of the linear terms and their respective coefficients represent either a main effect involving one variable or an interaction effect involving two or more variables. The regression equation for the determination of the actual and predicted values of the output respond of FAME yield for FrW and FW were given in Eq. (3) and (4), respectively.

\[ Y_{FrW} = -4140.63 + 64.35A + 25.94B + 20.56C - 20.87D - 0.47BD + 0.12CD - 0.61A^2 - 1.24B^2 - 0.40C^2 - 0.20D^2 \]  

\[ Y_{FW} = -6592.16 + 105.42A + 8.9B + 22.27C + 29.24D + 0.43AB - 0.06AC - 0.26AD - 0.08BC - 0.19BD + 0.02CD - 0.65A^2 - 1.30B^2 - 0.04C^2 - 1.11D^2 \]  

As shown in both equations, the positive coefficients indicated a linear effect, leading to increase of FAME yield. However, negative co-efficient term had negative effects that will decrease FAME yield. A positive sign in front of the equation indicates synergistic effect of the terms in enhancing FAME yield, whereas a negative sign indicates antagonistic effect of the terms in decreasing the yield [17-19].

Table 3 shows the analysis of variance (ANOVA), for response surface quadratic fitting for FrW and FW. The significance and fitness of the quadratic plot towards the effect of each significant terms and responses was determined. The statistical significance of the equation was evaluated by F-test and ANOVA for FrW and FW. It shows that the quadratic fitting was statistically significant at 95% confidence level ($\alpha = 0.05$) [17]. Furthermore, the significance of each regression coefficient and the interaction effect of each cross product are indicating by p-value. The smaller the p-value indicates greater significance of corresponding coefficient [18]. In this case, the values of “Prob > F” were less than 0.05 indicate that particular respond terms are statistically significant.

As evident in ANOVA analysis, the F-value for FrW and FW were attained at 30.94 and 39.65, respectively. Moreover, p-value 0.0001 for FrW and FW implies that the respond surface quadratic fitting is significant at 95% confidence level. For FrW, the p-value of 0.119 obtained is smaller than F-value of 3.51 at $\alpha = 0.05$. Likewise, FW also indicates a smaller p-value (p-value 0.2664 < F-value 2.31). Thus, it had sufficient evidence to conclude the

| Source | F-Value | P-Value | Prob > F | SD | R$^2$ | Adj. R$^2$ | F-Value | P-Value | Prob > F | SD | R$^2$ | Adj. R$^2$ |
|--------|---------|---------|----------|----|------|------------|---------|---------|----------|----|------|------------|
| Linear | 27.69   | 0.0027  | 0.0014   | 11.04 | 0.5529 | 0.4784     | 40.87   | 0.0053  | 0.0114   | 11.94 | 0.2795 | 0.1542     |
| 2FI    | 31.53   | 0.0022  | 0.0014   | 11.04 | 0.6429 | 0.4445     | 51.81   | 0.0038  | 0.0014   | 13.09 | 0.3605 | -0.016     |
| Quadratic | 3.51     | 0.119   | 0.9074   | 3.83 | 0.9687 | 0.9374     | 2.31    | 0.2664  | 0.9977   | 3.83 | 0.9776 | 0.9523     |
| Cubic | 7.66    | 0.0428  | 0.5111   | 11.04 | 0.9845 | 0.9277     | 2.56    | 0.2242  | 0.9929   | 11.04 | 0.9615 | 0.9615     |

Table 2. Summary of statistic for each response surface FrW and FW.
lack of fit testing for both quadratic responses are non-significant. Moreover, the "lack of fit F-value" attained for FrW and FW were 3.51 and 2.31, respectively and are relatively due to the pure error. In this study, non-significant lack of fit is ideal which indicated that the experimental data was satisfactorily fitted into the respond surface quadratic plot.

In addition, p-value lesser then 0.05 indicates ANOVA analysis indicate that the main respond surface variables has significant influence on FAME yield. In the case of FrW, the variables are A, B, C, BD, CD, A^2, B^2, C^2 and D^2. Meanwhile, for FW the significant variables that are influencing the yield of FAME are A, B, D, AB, AC, AD, BC, A^2, B^2, C^2 and D^2. However, values greater than 0.1000 indicate the respond terms are not significant.

The overall results obtained from the regression equation, determination co-efficient (R^2 >0.8) and p-value < 0.05 concludes that the responses were adequate for employing in this respond surface quadratic fittings for FrW and FW.

Table 3. ANOVA for response surface quadratic fitting for FrW and FW.

| Source        | Sum of Squares | df | Mean Square | F Value | p > F | FrW         | Sum of Squares | df | Mean Square | F Value | p > F |
|---------------|---------------|----|-------------|---------|-------|-------------|---------------|----|-------------|---------|-------|
| Block         | 352.27        | 1  | 352.27      |         |       |             | 27.99         | 2  | 13.99       |         |       |
| Model         | 6341.14       | 14 | 452.94      | 30.94   | < 0.0001 | 4449.35 | 317.81       | 39.65 | 0.0001 |
| A             | 2715.02       | 1  | 2715.02     | 185.47  | < 0.0001 | 391.59 | 391.59       | 48.86  | 0.0001 |
| B             | 306.03        | 1  | 306.03      | 20.91   | 0.0004  | 221.54 | 221.54       | 27.64  | 0.0002 |
| C             | 561.7         | 1  | 561.7       | 38.37   | < 0.0001 | 24.88  | 24.88        | 3.1    | 0.1015 |
| D             | 36.75         | 1  | 36.75       | 2.51    | 0.1354  | 634.67 | 634.67       | 79.19  | < 0.0001 |
| AB            | 0.56          | 1  | 0.56        | 0.038   | 0.8474  | 163.2  | 163.2        | 20.36  | 0.0006 |
| AC            | 49            | 1  | 49          | 3.35    | 0.0887  | 77.18  | 77.18        | 9.63   | 0.0084 |
| AD            | 1             | 1  | 1           | 0.068   | 0.7976  | 60.14  | 60.14        | 7.5    | 0.0169 |
| BC            | 0.3           | 1  | 0.3         | 0.021   | 0.8877  | 52.56  | 52.56        | 6.56   | 0.0237 |
| BD            | 196           | 1  | 196         | 13.39   | 0.0026  | 11.12  | 11.12        | 1.388  | 0.2599 |
| CD            | 342.25        | 1  | 342.25      | 23.38   | 0.0003  | 4.14   | 4.14         | 0.52   | 0.4850 |
| A^2           | 1477.84       | 1  | 1477.84     | 100.95  | < 0.0001 | 1784.81 | 1784.81      | 222.69 | < 0.0001 |
| B^2           | 782.25        | 1  | 782.25      | 53.44   | < 0.0001 | 941.42 | 941.42       | 117.46 | < 0.0001 |
| C^2           | 606.44        | 1  | 606.44      | 41.43   | < 0.0001 | 428.45 | 428.45       | 53.46  | < 0.0001 |
| D^2           | 148.31        | 1  | 148.31      | 10.13   | 0.0066  | 689.95 | 689.95       | 86.09  | < 0.0001 |
| Residual      | 204.94        | 14 | 14.64       |         |         | 104.19 | 104.19       | 8.01   |       |
| Lack of Fit   | 183.95        | 10 | 18.4        | 3.51    | 0.119   | 92.19  | 92.19        | 2.31   | 0.2664 |
| Pure Error    | 20.99         | 4  | 5.25        |         |         | 11.99  | 11.99        | 3.99   |       |
| Cor. Total    | 6898.35       | 29 |             |         |         | 4581.53 |             |        |       |

3.2. Effects of Interaction between Variables on FAME Yield

Transesterification on lipid derived from larvae of *H. illucens* using sulfuric acid catalyst and methanol was investigated. The effects of reaction temperature, methanol to sample mass ratio, reaction time and catalyst loading on FAME yield were investigated using design expert software. In statistical analysis, effects of interactions among variables were considered as the interaction is essential for comprehensive understanding of a process [18-19].

3.2.1. Effect of temperature and methanol to sample mass ration FAME yield

The plot in Fig. 3(a) and (b) shows the effect of varying temperature and methanol to sample mass ratio while the catalyst concentration were held at constant. For FAME yield derived from fruit waste, the yield was found to be lowest at a lowest temperature of 45°C with the lowest methanol to mass ratio of 3:1. However, the yield began to decrease with further increased in reaction temperature and methanol to sample mass ratio up to maximum range of 55°C and 9:1, respectively. As shown in Fig. 3(a), reaction temperature around 50-52°C and methanol to sample mass ratio within the range of 6:1 to 6.5:1 showed the maximal yield was approximately 92%.

In the meantime, the yield observed in Fig. 3(b) was found to be lowest at the minimum reaction temperature
and methanol to mass ratio at 65°C and 3:1, respectively. It was observed that the yield was found to increase with reaction temperature and methanol to sample mass ratio up to its center point. As shown in this Fig. 3(b), the maximal yield attained was approximately 90 % at reaction temperature ranging approximately 70 to 72°C and methanol to sample mass ratio in the range of 6:1 to 7:1. However, the yield began to decrease with further increased in reaction temperature at 72°C and methanol to sample mass ratio at 7:1 onwards. Comparatively, the yield was found to increase with reaction temperature and methanol to sample mass ratio up to its center point for both fruit waste and food waste. Lower reaction temperature was required for FAME production derived from fruit waste (50-52°C) as compared to food waste (70 to 72°C).

The interrelation between temperature and methanol to sample mass ratio elucidate the optimal value of methanol to sample mass ratio for the yield. From this study, it was observed that high or low amounts of methanol to sample mass ratio will have negative impact on FAME yield. This can be justified by the circumstance that the transesterification is a reversible reaction and thus, excess of methanol will on the other hand facilitate the reaction towards increasing yield while maintaining the reaction at its equilibrium state [18-19]. On the other hand, if the given methanol ratio is exceeding its equilibrium state; it will decrease the yield. Similar findings were also reported by [17, 20]. On the contrary, insufficient amount of methanol to sample mass ratio led to an incomplete reaction. Therefore, both temperature and methanol to sample mass ratio contribute an important role to obtain optimal value.

Fig. 3(a). Effects of temperature (A) and methanol to mass ratio (B) on the yield of FAME derived from fruit waste.

Fig. 3(b). Effects of temperature (A) and methanol to mass ratio (B) on the yield of FAME derived from food waste.

3.2.2. Effect of temperature and catalyst on FAME yield

The interaction between temperature (A) and catalyst (D) on the FAME yield was illustrated in Fig. 4(a) and (b). As illustrated in Fig. 4(a), FAME yield increased tremendously with the increase of catalyst loading between 15 to 20 v/v% and steadily increase leading nearly plateau between 21 to 22 v/v%. At a fixed level of catalyst loading, Fig. 4(a) appears to be insignificant change in methyl ester yield with increasing reaction temperature, within the range set. This could explicate by the high p-value of 0.1354 for catalyst loading in the response surface plot, signifying the insignificance of this variable. From the observations, the methyl ester yield shows a tremendous increased in reaction temperature from 45 to 50°C. However, the yield decreases at elevated temperature at 53°C onwards and catalyst loading of above 22 v/v%. Similar trend was observed in Fig. 4(b), when both the temperature and the catalyst increase beyond optimum the FAME yield starts to decline. Lower catalyst loading (6.5 v/v%) and higher reaction temperature (70°C) were required to achieve better FAME yield derived from food waste. The result obtained was further explicate by the significant p-value (<0.0001) attained for both catalyst loading and temperature.

Fig. 4(a) and (b). Effects of temperature (A) and catalyst loading (D) on the yield of FAME derived from food waste.
Comparing with both Fig. 3 and 4, FAME yield was sensitive to reaction temperature, methanol molar ratio and catalyst. Hence, the amount of catalyst used in this study had an effect on the yield of FAME. The 3D response obtained were elliptical signifying that there were distinct optimum operating conditions. The reduction of yield could be due to the excess of catalyst loading along with the increasing of reaction temperature that led to the formation of soaps via saponification reaction. This formation of soaps was observed during the purification process of FAME. Similar findings were observed while comparing with Mostafaei et al. [19] and Vicente et al. [21] studies, which high catalyst loading will favor the saponification reaction and can intensify the emulsification of ester and glycerol that led to complication in FAME separation process. Hence, this phenomenon will contribute to the reduction on the yield of FAME.

Moreover, excess of catalyst would intensify the formation of esterification of free fatty acids and give rise to water formation. The excess water formed will cause hydration and would deactivate the acidic hydroxyl groups during the reaction [18]. Thus, results in lower FAME yield.

3.2.3. Effect of temperature and reaction time on FAME yield

Figures 5(a) and (b) illustrate the 3-D respond surface plots, indicating an interrelation interaction between the independent variables in reaction temperature (A) and reaction time (C) with FAME yield. As observed, increased in reaction temperature along with the increase in reaction time gave rise to methyl ester yield. This can be seen from Fig. 5(a), FAME yield is minimal with lowest temperature of 45°C and minimum reaction time of 240 min; however, the methyl ester yield increased with increasing reaction time and temperature. The highest methyl ester yield obtained was at 50°C and 255 min with the yield of approximately 95%. Once the reaction temperature and reaction time was greater than its center point value, the reverse trend was observed. As observed, lower reaction temperature of 45°C did not favour the transesterification process. In our point of view, reaction temperature of 45°C and above is required to initiate the reaction to occur.

On the contrary, the highest methyl ester yield obtained for FAME derived from food waste was at higher temperature (71°C) than that of fruit waste with the yield of approximately 91%. However, further increased in the reaction temperature (above 71°C) and reaction time (above 250 min), the reverse trend was observed. In our point of view, the reaction time and temperature is very much depending on the nature of the fatty acids origin as well as the properties of the catalyst either from biocatalyst or chemical catalyst. For instance, the reaction time required to achieve highest yield of 96.97% employing Novozym 435 was 4 times greater than that in this study [22].
In summary to both RSM analysis explained in section 3.2.1 to 3.2.3, an interesting point can be noted that the range of variables is not always the same despite producing from the same feedstock. For example, the reaction temperatures for in-situ transesterification of the lipids of pre-pupae fed with fruit waste were lower than that of food waste. The optimum reaction temperatures required for pre-pupae fed with fruit waste and food wastes were at 51 and 70°C, respectively. Furthermore, the amount of catalysts and methanol to sample mass ratio used in both optimizations were also different. The amount of catalyst used to transesterify the lipids derived from fruit waste was 2 fold higher as compared to the same catalyst used for transesterifying the lipid derived from food waste. This difference may be due to different feeds acquired by the *H. illucens* during larvae stage. Therefore, the yields of FAME produced are depending upon the fatty acid contents and its compositions metabolized by the larvae. Table 4 compares the optimum parameters for FAME production based on various insects.

| Types of insect | Catalyst | Reaction time | Reaction temperature | Solvent to oil/sample ratio | FAME yield | Ref |
|----------------|----------|---------------|----------------------|-----------------------------|------------|-----|
| *H. illucens* prepupae (Fruit waste feed) | H$_2$SO$_4$; 15v/v% | 253 min | 51°C | 8:3:1 | 96.15% | This study |
| *H. illucens* prepupae (Food waste feed) | H$_2$SO$_4$; 7v/v% | 254 min | 71°C | 6.8:1 | 94.63% | This study |
| Yellow mealworm beetle | NaOH, 0.8 w/w % | 30 min | 65°C | 6:01 | 96.80% | [25] |
| *Zophobas morio* larva | KOH, 1.25 wt % | 45 min | 60°C | 5:01 | 94.63% | [24] |
| Black soldier fly larvae | Protamex, 3.85% | 4.27 h | 38.1°C | 4.33:1 | 92.35 wt % | [23] |
| Black soldier fly larvae (SRF feed) | Novozym 435, 17.8% | 1.2 h | 39.5°C | 14.64:1; 6.1 | 96.97% | [22] |
| Black soldier fly larvae (SRF feed) | 1% H$_2$SO$_4$ (pre-treatment); 0.8w/w% NaOH (alkaline transesterification) | 30 min | 75°C (pre-treatment); 68°C (transesterification) | 6:1 (pre-treatment); 4:1 (transesterification) | 96.90% | [8] |

Table 4. Comparison of reaction parameters based on various insect for biodiesel production.
4. Optimization of Response Variables

The optimization of specific response was accomplished to achieve the optimal reaction conditions of yield based on the particular developed mathematical equations. Each reaction variable was set in range and the yield was set maximized. Two sets of RSM analysis were carried out using in-situ transesterification of lipid of pre-pupae derived from fruit waste and food waste.

The first set of the response surface analysis was conducted using the lipids of pre-pupae derived from fruit waste. The optimal yield obtained was 96.15 % based on the optimum conditions of reaction temperature of 51°C; methanol; mass ratio of 8.3:1; reaction time of 253 min and catalyst loading of 15.1 v/v%.

The optimal yield obtained from the second set of response surface analysis was 94.63 % using lipids of pre-pupae derived from food waste. The optimum conditions for reaction temperature was 71°C, with methanol to mass ratio of 6.8:1, at reaction time of 254 min and catalyst loading of 7.0 v/v%. It was observed that the yield from both optimization were almost the same from those obtained by Zheng et al. [8-9] which attained at 96.9 % fed with solid restaurant food waste via 2 step reaction which is acid catalyzed esterification and alkaline catalyzed transesterification at 75 and 65 °C, respectively. The optimal value of input process parameters was given in Table 5.

Table 5. Optimization conditions for maximal FAME yield.

| Variable                  | Goal       | FrW | FW |
|---------------------------|------------|-----|----|
| Temperature, °C           | Is in range| 51  | 71 |
| MeOH : sample mass ratio  | Is in range| 8.3:1| 6.8:1|
| Reaction time, min        | Is in range| 253 | 254|
| Catalyst loading, v/v%    | Is in range| 15  | 7  |
| FAME yield, %             | Maximized  | 96.15| 94.63|

4.1. Biodiesel Properties Analysis

The optimized yield of FAME was further analyzed for biodiesel properties according to EN14110 and ASTM D6584. The properties of produced biodiesel had a reasonable agreement with the standards. The results shows that, FAME produced from H. illucens prepupae contains 99.85 % of ester content, a minimum value of < 0.1wt% of sulphated ash and sulphur S15 grade. Besides that, the residual carbon content detected in 100% of FAME was < 0.1wt%. Kinematic viscosity is an important property for fuel atomization and distribution, which was attained at 5.4 cst was within the range of standards. The biodiesel cloud points were considerably higher than petro-diesel due to fatty acid composition of the prepupae, which consist lauric acid. Copper strip corrosion was rated at 1a and the oxidation stability was achieved at 17 hours was in accordance with the standards.

5. Conclusions

Based on the findings, following conclusions were made with regards to the objectives of this study. In summary, an understanding was gained based on the optimization. The optimum yields of biodiesel were affected by the variables such as catalyst concentration, alcohol to molar ratio, reaction time and temperature. An increase or decrease of either one of these variable will eventually affect the yield of biodiesel. Thus, it provides the understanding on how these variables can influence the process and ultimate measures to be taken in order to achieve optimum. The optimization of the reaction variables affecting the yield had been achieved based on Box Behnken experimental design for lipids of pre-pupae derived from fruit waste and food waste. The first set of the optimization was conducted using the lipids of pre-pupae derived from fruit waste. The optimal yield obtained was 96.15 % based on the optimum conditions of reaction temperature of 51°C; methanol: mass ratio of 8.3:1; reaction time of 253 min and catalyst loading of 15.1 v/v%. Furthermore, the optimal yield obtained from the second set of optimization using lipids of pre-pupae derived from food waste was achieved at 94.63 %. The optimum conditions for reaction temperature was 71°C, with methanol to mass ratio of 6.8:1, at reaction time of 254 min and catalyst loading of 7.0 v/v%. Comparatively, the yield from both optimization were almost the same from those obtained by Zheng et al. [8-9] which attained at 96.9 % fed with solid restaurant food waste.

From the findings, it is conclude that FAME derived from pre-pupae lipids are feasible to be used for biodiesel. This study is to pave way for sustainable waste management where by the larvae are used as an agent to convert the waste and at the same time up-cycle to higher value product such as biodiesel. Therefore, this will be able to solve both organic waste and energy issue.

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Leong Siew Yoong is currently an Assistant Professor at Faculty of Engineering and Green Technology, University Tunku Abdul Rahman (Kampar campus). She obtained both MSc. (Chemical Engineering) and PhD (Environmental) at Universiti Teknologi PETRONAS. Her MSc. studies were related to the “Development of copper-doped TiO₂ photocatalyst for hydrogen production under visible light”. In 2011, she embarked on her PhD research related to “Upcycling of organic waste into biodiesel production via Hermetia illucens larvae”. Her research interests are catalysis, bioprocessing and bioconversion of waste to biofuel, biofertilizer and feed.

S. R. M. Kutty, photograph and biography not available at the time of publication.

Mohammed J. K. Bashir, photograph and biography not available at the time of publication.

Qunliang Li, photograph and biography not available at the time of publication.