Optimisation and characterisation studies of biodiesel production from black soldier fly larvae fed by soya residue

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Abstract. In this study, a two-step transesterification process was applied to convert crude BSF larval oil into biodiesel. The effects of methanol:oil (molar ratio), catalyst amount (weight %), reaction time (minutes) and temperature (°C) on biodiesel production were investigated. Central Composite Design (CCD) of Response Surface Methodology (RSM) was used to optimise experimental data obtained from these four variables. The prediction of biodiesel yield was made by employing a quadratic model, whereby the R² values were greater than 0.99. Based on optimisation studies, a combination of an application of petroleum ether and reaction time of 6 hours was the best to extract crude larval oil with 47% of lipid yield. Meanwhile, methanol:oil molar ratio of 12:1, catalyst (NaOH) concentration of 1.0 weight %, a reaction time of 32 minutes and temperature of 60°C were required to obtain biodiesel with 96% of production yield from BSF crude larval oil. Both BSF crude larval oil and biodiesel were characterised using several analytical instruments such as Fourier Transform Infrared (FTIR) spectrometer and Gas Chromatography – Flame Ionisation Detector (GC-FID). Overall, findings from this study highlight the potential to produce eco-friendly biodiesel from BSF larvae reared on soya residue.

1. Introduction
Fossil fuel is the dominant world energy supply. However, due to the disadvantages of the non-renewable energy resources, the interest in investigating the alternative energy resources known as renewable energy keeps on increasing. Moreover, Soliman et al. [1] stated that renewable energy provides energy security as compared to non-renewable energy as well to promote global energy growth. The renewable energy including solar, hydro, wind, tidal and biomass provides sustainable and environmentally friendly characteristics than non-renewable energy [2]. Biodiesel is one of the potential renewable energies that has been widely reported by researchers and received high consideration due to several advantages such as engine combustion efficiency without engine modification, low sulphur content, high cetane number and biodegradable [3-5]. Moreover, application of biodiesel is also able to reduce the greenhouse hazardous gas emission mainly from fuel combustion such as carbon dioxide, methane, nitrous oxide, ozone and chlorofluorocarbons [6,7].
Biodiesel is a type of diesel fuel derived from the renewable lipid of plants or animals and consists of long-chain fatty acid esters [8]. Vegetable oil is the most famous feedstock category for biodiesel production worldwide. However, the use of vegetable oil as a biodiesel feedstock is restricted and minimal in many countries due to the high total biodiesel production cost [9]. This situation occurred due to the increment of raw material cost for about 60-75% of the total biodiesel production and also the requirement of large arable plant for extensive biodiesel production [10,11]. Additionally, the usage of vegetable oils particularly edible oils may cause a food crisis in the long term [9]. Another issue is the seasonal harvest period for almost oilseeds which make vegetable oils are inefficient for the biodiesel production process [12]. Previous studies by Zheng et al. [11], Nguyen et al. [12], Wong et al. [13] and Ishak et al. [14] have shown that insect larvae can be used as alternative feedstocks for biodiesel production. Although they have reported the success to produce biodiesel from crude larval oil, the influence and optimisation of several crucial experimental parameters on esterification and transesterification processes have been seldom studied statistically. Therefore, this work was devised with an ultimate and novel aim to apply CCD of RSM for optimisation of biodiesel production from BSF larvae reared on soya residue for the first time.

2. Materials and methods

2.1. Cultivation of BSF larvae

A colony of adult BSF (Hermetia illucens) was placed in a rearing cage with a size of 150 cm x 75 cm. Soya residue and water were provided in the rearing cage to promote BSF for mating and oviposition process. Soya residue was collected from a food and beverage factory in Rawang, Selangor, Malaysia. The temperature and humidity were kept in the range of 25-32°C and 60-75%. After 20 days, BSL larvae were harvested, rinsed, and inactivated at 100°C for 5 minutes followed by drying in an oven at 60°C for 24 hours.

2.2. Extraction of crude BSF larval oil

About 25 g of dried BSF larvae were placed in three-round bottom flasks containing 250 mL of petroleum ether, ethanol and acetone, separately. The extraction was carried out in a Soxhlet system and set from 1 to 6 hours. Following extraction, the distillation process was performed to remove excess solvent and to obtain crude BSF larval oil. The crude oil was then heated in an oven at 105°C until a constant weight was achieved. It was also treated with 1% (v/v) of concentrated phosphoric (85%, v/v) and 2-4% of softened water at 30°C. Finally, the crude BSF larval oil was centrifuged and high purity of BSF larval oil was collected after the refinement process. The percentage of crude BSF larval oil produced was determined.

2.3. Production of crude larval biodiesel

In this study, crude BSF larval oil was converted to biodiesel through a two-step transesterification process, which involved acid-catalysed esterification and alkaline-catalysed transesterification [15,16]. The acid-catalysed esterification step was important to reduce the FFA content in the crude oil, meanwhile, alkaline-catalysed transesterification converted the esterified oil to biodiesel. For acid-catalysed esterification procedure, about 15 g of BSF larval oil was placed into a 250 mL of a conical flask, heated to 45°C and added with the mixture solution of methanol and sulphuric. The resulted solution was then stirred for 60 minutes followed by centrifuge at 400 rpm for 15 minutes. The upper layer (esterified oil) was then transferred to a conical flask and dried in an oven at 105°C for 15 minutes. The alkaline-catalysed transesterification was conducted by placing about 15 g of esterified oil into a conical flask, heated to 45°C, added with the mixture solution of methanol and sodium hydroxide and stirred for 15 minutes. The solution was then separated using a centrifuge at 400 rpm for 10 minutes. The upper layer (biodiesel) was collected and the percentage yield was determined.
2.4. Statistical analyses
A five-level four-factor CCD was employed and generated thirty experimental runs. Statistical analyses aimed to determine the optimum biodiesel yield (Y) as well as to investigate the effect of experimental variables, namely methanol:oil molar ratio (A), catalyst amount (B), time (C) and temperature (D) on biodiesel production. The experimental data were analysed using RSM and their fitness to the quadratic polynomial equation was examined. The statistical analyses such as analysis of variance (ANOVA) and regression coefficient ($R^2$) were accomplished based on the response obtained.

2.5. Characterisation studies
In this study, several analyses were carried out to characterise the physical and chemical properties of crude BSF larval oil and BSF biodiesel. The analyses were performed using a Thermo Nicolet 6700 FTIR Spectrometer, a Jeol JNM-ECX-500 NMR Spectrometer and a Shimadzu GC-17A GC equipped with a Zebron ZB-FAME column and an FID.

3. Results and discussion

3.1. Monitoring of BSF larvae growth
Observation of BSF larval growth development was conducted for 20 days of the cultivation process. Table 1 presents the physical properties of BSF larvae reared on soya residue. Four main physical properties were determined including colour, individual weight, length and width. The BSF larval weight increased up to day 15 of the cultivation process. However, the reduction of BSF larval weight about 14.7% was observed at day 20 as compared to day 15 of cultivation. A similar finding was reported by Wong et al. [14], of which the weight decreased from 5.24 g to 3.70 g for fifth to six instars of BSF larvae.

| Properties          | Cultivation (days) |
|---------------------|--------------------|
|                     | 5                  | 10                 | 15                 | 20                 |
| Colour              | Creamy             | Beige              | Dark beige         | Black              |
| Individual weight (g)| 0.0988 ± 0.15      | 0.1414 ± 0.17      | 0.2081 ± 0.26      | 0.1775 ± 0.15      |
| Length (mm)         | 14.00 ± 0.58       | 17.67 ± 0.33       | 21.33 ± 0.42       | 19.67 ± 0.28       |
| Width (mm)          | 3.67 ± 0.19        | 4.78 ± 0.33        | 5.87 ± 0.26        | 4.56 ± 0.21        |

Values represent mean of three replicates ± standard error.

This scenario was mainly due to the cessation or commonly known as terminal growth period that correlated to the metamorphosis of insect larval life cycle [17]. In this study, changes of BSF larval colour could be observed from creamy to black during the cultivation of BSF larvae within 20 days. Turning of insect larval colour was significantly in accordance with the metamorphosis process [18]. According to Janssen et al. [19], the variations of BSF larval colour was due to iron-polyphenol complexes content in the BSF larvae itself.
3.2. Extraction of crude BSF larval oil

Solvent extraction is a widely used method for the extraction of crude oil. In this study, the effectiveness of lipid extraction was evaluated using three solvents with different polarity properties, namely petroleum ether, acetone, and ethanol. Figure 1 shows the percentage of lipid yield extracted from BSF larvae using the aforementioned solvents. It is apparent that ethanol has resulted in the highest yield of BSF lipid of up to 47%, followed by petroleum ether (44%) and acetone (43%). As reported by López et al. [20], a non-polar solvent such as petroleum ether results in slightly lower yield than ethanol due to its ability to extract triglycerides only. Meanwhile, acetone also has potential in extracting lipids but from this experiment, the application of acetone produced the lowest crude oil yield as compared to other solvents. This outcome was consequently due to polarity properties of acetone itself that was more non-polar than ethanol and less non-polar than petroleum ether. Indeed, polar and non-polar lipids dissolve in acetone but not as high as ethanol and petroleum ether.

![Figure 1. Lipid extraction from BSF larvae using several solvents.](image)

Reaction time has a significant influence on the extraction of crude oil. From figure 1, it is clear that when extraction time was increased, the lipid yield (%) was also increased but with a marginal increment. For example, the percentage yield of lipid extracted by ethanol after 4, 5 and 6 hours of extraction was determined as 42, 44 and 47%, respectively. As discussed by Feng et al. [21], the marginal increment trend might be due to washing and diffusion phases involved in the extraction process. In the context of crude larval oil extraction, washing phase refers to the cleaning of lipid on the insect body surface while the diffusion phase refers to mass transfer of solid-liquid from lipid to extractant solvent. Although ethanol extracted the highest lipid yield, petroleum ether was more favourable to be used as an extractant solvent due to its ability to extract triglycerides only. Therefore, the optimum extraction process was set with a combination of petroleum ether and 6 hours of extraction time.

3.3. Optimisation of transesterification

The design matrix for 30 experiments and experimental yields using CCD are shown in Table 2. From Table 2, it is clear that the biodiesel yield was significantly influenced by the combination of each variable. The experimental error and reproducibility of obtained data were indicated by the center point (five replicates).

Table 3 presents an analysis of variance (ANOVA) for the CDD model, which is important for verification of the significance and fitness of the quadratic model. The F-value and Prob>F values for model were determined as 13.20 and <0.0001, respectively, implying that the model was significant at 95% confidence level. ANOVA results identified that A, B, C, D, AB, AC, AD, BC, BD, CD, A^2, B^2, C^2, and D^2 were significant model terms. The lack of fit F-value of 4.71 and p-value of 0.0506 indicates that the model was satisfactorily fitted to experimental data.
The \( R^2 \) value of 0.9890 represents the reasonable agreement between the predicted yield and the actual yield. The relationship for predicted yield against actual yield is shown in figure 2 (a), while normal probability against studentised residuals and studentised residuals against run number are shown in figures 2 (b,c). Adequate precision (AP) is defined as a measurement of signal to noise ratio, whereby the range of predicted values at the design points are compared with the average prediction error. The AP value obtained from this study was determined as 34.764 (table 3) which indicates a desirable ratio. An AP value which greater than 4 suggests adequate model discrimination [22].

3.3.1. Effect of methanol:oil molar ratio
The 3D plots of the interaction effect between A: methanol:oil, B: catalyst amount, C: time and D: temperature on biodiesel yield are presented by figures 3 (a,b,c). The plots show that high biodiesel yield (>90%) can be obtained with methanol:oil molar ratio between 8:1 and 10:1. In general, a further increment in methanol:oil molar ratio from 11:1 to 12:1 might reduce biodiesel production [23].

3.3.2. Effect of catalyst amount
The application of catalyst in biodiesel production will accelerate the breaking of triglycerides (TAG) bond during the transesterification process [24]. Figures 3 (a,d,e) present the interaction of catalyst amount between A: methanol:oil, C: time and D: temperature on biodiesel production. Based on 3D plots, high biodiesel production (>90%) can be obtained when the amount of catalyst (weight %) in the range from 0.5 to 1.0. However, Rajan et al. [25] have reported that high catalyst amount may lead to soap formation and therefore difficult to separate glycerol from biodiesel.

| Run | Methanol:oil (molar ratio) | Catalyst amount (%) | Time (min) | Temperature (°C) | Biodiesel yield (%) | Run | Methanol:oil (molar ratio) | Catalyst amount (%) | Time (min) | Temperature (°C) | Biodiesel yield (%) |
|-----|---------------------------|---------------------|------------|-----------------|---------------------|-----|---------------------------|---------------------|------------|-----------------|---------------------|
| 1   | 10:1                      | 1.5                 | 40         | 45              | 55                  | 16  | 10:1                      | 1.5                 | 20         | 45              | 55                  |
| 2   | 14:1                      | 1.5                 | 40         | 45              | 59                  | 17  | 12:1                      | 1.0                 | 30         | 75              | 67                  |
| 3   | 10:1                      | 0.5                 | 20         | 65              | 65                  | 18  | 12:1                      | 1.0                 | 30         | 55              | 83                  |
| 4   | 10:1                      | 0.5                 | 40         | 65              | 73                  | 19  | 10:1                      | 0.5                 | 40         | 45              | 44                  |
| 5   | 12:1                      | 1.0                 | 30         | 55              | 89                  | 20  | 12:1                      | 1.0                 | 30         | 35              | 45                  |
| 6   | 10:1                      | 0.5                 | 20         | 45              | 35                  | 21  | 12:1                      | 1.0                 | 30         | 55              | 90                  |
| 7   | 8:1                       | 1.0                 | 30         | 55              | 45                  | 22  | 14:1                      | 0.5                 | 20         | 65              | 68                  |
| 8   | 12:1                      | 1.0                 | 30         | 55              | 90                  | 23  | 14:1                      | 1.5                 | 20         | 65              | 50                  |
| 9   | 12:1                      | 1.0                 | 50         | 55              | 58                  | 24  | 14:1                      | 0.5                 | 40         | 45              | 51                  |
| 10  | 12:1                      | 1.0                 | 30         | 55              | 93                  | 25  | 12:1                      | 1.0                 | 15         | 55              | 64                  |
| 11  | 12:1                      | 2.0                 | 30         | 55              | 65                  | 26  | 14:1                      | 0.5                 | 20         | 45              | 46                  |
| 12  | 10:1                      | 1.5                 | 20         | 65              | 69                  | 27  | 16:1                      | 1.0                 | 30         | 55              | 57                  |
| 13  | 10:1                      | 1.5                 | 40         | 65              | 78                  | 28  | 14:1                      | 1.5                 | 40         | 65              | 50                  |
| 14  | 14:1                      | 1.5                 | 20         | 45              | 61                  | 29  | 12:1                      | 0.2                 | 30         | 55              | 54                  |
| 15  | 14:1                      | 0.5                 | 40         | 65              | 78                  | 30  | 12:1                      | 1.0                 | 30         | 55              | 90                  |

3.3.3. Effect of reaction time
Figures 3 (b,d,f) depict the results for interaction study between reaction time with A: methanol:oil, B: catalyst amount and D: temperature on biodiesel yield. It is apparent that high biodiesel production (>90%) could be obtained when the reaction was conducted between 40 to 60 minutes. Extending the reaction time to 80 minutes with increasing catalyst amount up to 1.5 weight % would cause a significant reduction in biodiesel yield. As discussed by Kafuku and Mbarawa [26], acceleration of ester hydrolysis and saponification are likely to occur at a longer reaction time. In contrast, a different trend was obtained for interaction between reaction time with methanol:oil and temperature. The high biodiesel yield (>90%) may be achieved at reaction time between 60 to 80 minutes. This might be due to insufficient reaction time to reach equilibrium (figures 3 (b,f)).
Table 3. Analysis of variance (ANOVA) for CDD model.

| Source       | Sum of squares | Degree of freedom | Mean of square | F-value | Prob>F   |
|--------------|----------------|-------------------|----------------|---------|----------|
| Model        | 7036.32        | 14                | 502.59         | 13.20   | < 0.0001 |
| A-Methanol:oil | 7.04         | 1                 | 7.04           | 0.18    | 0.6733   |
| B-Catalyst amount | 126.88    | 1                 | 126.88         | 3.33    | 0.0879   |
| C-Time       | 110.96        | 1                 | 110.96         | 2.91    | 0.1084   |
| D-Temperature | 1190.04      | 1                 | 1190.04        | 31.26   | < 0.0001 |
| AB           | 248.06        | 1                 | 248.06         | 6.52    | 0.0221   |
| AC           | 10.56         | 1                 | 10.56          | 0.28    | 0.6061   |
| AD           | 280.56        | 1                 | 280.56         | 7.37    | 0.0160   |
| BC           | 39.06         | 1                 | 39.06          | 1.03    | 0.3271   |
| BD           | 517.56        | 1                 | 517.56         | 13.60   | 0.0022   |
| CD           | 14.06         | 1                 | 14.06          | 0.37    | 0.5524   |
| A²           | 2067.81       | 1                 | 2067.81        | 54.32   | < 0.0001 |
| B²           | 1134.23       | 1                 | 1134.23        | 29.79   | < 0.0001 |
| C²           | 1168.78       | 1                 | 1168.78        | 30.70   | < 0.0001 |
| D²           | 1510.11       | 1                 | 1510.11        | 39.67   | < 0.0001 |
| Residual     | 571.21        | 15                | 38.07          |         |          |
| Lack of fit  | 516.21        | 10                | 51.62          | 4.71    | 0.0506   |
| Pure error   | 54.83         | 5                 | 10.97          |         |          |
| Cor total    | 7607.37       | 29                |                |         |          |

R²: 0.9885  Adjusted R²: 0.9890  Adequate precision: 34.764

**Figure 2.** Residual plots (a) predicted against actual plot, (b) normal probability against studentised residuals and (c) studentised residuals against the run number.
3.3.4. Effect of reaction temperature

Figures 3 (c,e,f) show the interaction between reaction temperature and A: methanol:oil, B: catalyst amount, and C: time on biodiesel yield. From 3D plots, it was clear that high biodiesel yield (>90%) could be obtained between the reaction temperature of 55 and 65°C. A higher reaction temperature of 75°C reduced biodiesel production, which was due to vaporisation of methanol. Furthermore, there was a possibility of side reaction of alkyl ester hydrolysis to occur and to produce acids at the higher temperature [27].

![3D plots](image)

Figure 3. 3D plots represent the interaction between each variable on biodiesel yield: (a) catalyst amount and methanol:oil, (b) time and methanol:oil, (c) temperature and methanol:oil, (d) time and catalyst amount, (e) temperature and catalyst amount, and (f) temperature and time.

3.4. Optimisation of biodiesel production

The maximum biodiesel yield was determined by optimisation study. In this study, biodiesel yield was set to a maximum value, while other variables were set in a range between high and low levels. The optimum biodiesel yield of 95.7% was achieved by the transesterification of BSF larval oil with methanol:oil molar ratio of 12:1, catalyst amount of 1.0 weight %, a reaction time of 32 minutes and
reaction temperature of 60°C. RSM with CCD was able to predict the optimisation of biodiesel production and transesterification process with a small percentage error of 1.01%.

3.5. FTIR analysis
FTIR analysis is important to confirm the functional groups that are present in the BSF larval lipid and biodiesel. Figure 4 shows an overlay of FTIR spectra of BSF larval lipid and biodiesel cultivated in soya residue. The absorption bands observed at 2920 and 2851 cm\(^{-1}\) can be related to stretches of C-H (alkanes) and sharp bands at 1740 to 1741 cm\(^{-1}\) can be assigned to C=O stretches (ketones, carboxylic acid and esters) [28]. The absorption bands observed at 1165, 1149, 1116 and 1111 cm\(^{-1}\) can be ascribed to C-O stretches (ester). The peaks at 721 and 720 cm\(^{-1}\) can be related to C-H bending vibration (long-chain fatty acids) [29]. In the context of figure 4 (b), the –CH\(_3\) and –CH\(_2\) bending vibrations of triglycerides were identified at 1456, 1435 and 1374 cm\(^{-1}\) [30]. Overall, results obtained from FTIR analysis confirmed the formation of fatty acid methyl esters (FAMEs) in BSF larval biodiesel derived from larval lipid cultivated on soya residue.

![Figure 4. FTIR spectra of crude lipid (a) and biodiesel (b) produced from BSF larvae fed by soya residue.](image)

3.6. GC-FID analysis
In this study, the fatty acid composition of BSF larval biodiesel was analysed using GC-FID. In general, GC-FID was mainly applied for determining the degree of saturation and unsaturation of biodiesel. Figure 5 displays the GC-FID chromatogram of the larval biodiesel produced from BSF larvae fed by soya residue. As can be seen from figure 5, eight FAMEs were detected in the BSF larval biodiesel including lauric acid (C12), myristic acid (C14), palmitic (C16), palmitoleic acid (C16:1), stearic (C18), oleic acid (C18:1), linoleic acid (C18:2) and gondoic acid (C20:1). It is evident from GC-FID analysis that BSF larval biodiesel comprised of saturated (lauric acid, myristic acid,
palmitic acid, capric acid and stearic acid) and unsaturated (oleic acid, palmitoleic acid, linoleic acid and gondoic acid) FAMEs.

![Figure 5. GC-FID chromatogram of biodiesel produced from BSF larvae fed by soya residue.](image)

4. Conclusion
RSM with CCD based on several experimental parameters was successfully employed for the optimisation of biodiesel production from BSF larvae fed by soya residue. At optimum conditions of 12:1 (methanol:oil molar ratio), 1.0 weight % catalyst amount within 32 minutes of reaction time and 60°C of reaction temperature, 95.7% of biodiesel was produced. The physicochemical properties of crude BSF larval oil and biodiesel were characterised using FTIR spectrometer and GC-FID. The FTIR analysis revealed the appearance of absorption bands represent the stretches of C-H, C=O and C-O which confirmed the formation of fatty acid methyl esters in BSF larval biodiesel. Meanwhile, GC-FID proved that BSF larval biodiesel consists of saturated and unsaturated of fatty acid methyl esters.

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