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Exploring the potential of lipids from black soldier fly: New paradigm for biodiesel production (I)



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ABSTRACT

Black soldier fly, a high lipids containing insect, can be used as a new and viable biomass feed-stock, using organic matters (animal manure, restaurant waste, and fermentation straw) and increasing the overall biodiesel yield. This study proposed microwave extraction method to extract lipid from the insect for biodiesel production. The factors influencing the extraction yield % (w/w) were discussed in detail. Response surface methodology was used to investigate the effect of extraction conditions on the lipid extraction yield. The results obtained by statistical analysis showed that the quadratic model fits in the cases. Gas chromatography mass spectra of the obtained insect lipid revealed 22.54% oleic, 12.67% linoleic, and 6.45% palmitoleic acid in its composition. These compositional data were qualitatively confirmed with Fourier transform infrared (FTIR), thermal gravimetric (TG) and differential scanning calorimeter (DSC) analyses of extracted lipid sample. The comparison of the insect fatty acids profile from the energy insect black soldier fly larvae with common biodiesel feed-stocks showed suitability of the insect for production of biodiesel.

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1. Introduction

The energy demand from fossil fuels for transportation has been increasing during the last few years, and it will be the strongest growing energy demand sector in the future [1,2]. However, the expected depletion of fossil fuels and the environmental problems associated with their combustion limit their utilization in the future [3]. Therefore, it has been realised that both exploration of new energy sources and safety of environment are equally important for sustainable development. One of the most promising renewable fuels proposed as an alternative is biodiesel that can be directly used with current engine and refuelling technology [4]. As sources of first generation biofuel, the competitive potential of biodiesel is limited due to high cost of common lipid feedstocks (soybean, canola, rapeseed, sun-flower, palm, and coconut oils), which constitutes 70–85% of the overall biodiesel production cost, strongly influencing the final price of this biofuel [5]. Recently,

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microalgae have gained much attention as a promising resource for biodiesel production. However, the major obstacle for commercialization of biofuels obtained from microbes is still the high production cost involved [6,7]. Most studies use autotrophic microalgae, however they exhibit a slower growth rate [8]. Additionally, autotrophic cultures cannot achieve high biomass concentrations and high oil productivities, due to light and oxygen limitations [9]. Therefore, it is crucial to explore approaches to reduce the costs of microbial biofuel production processes, by using low-cost raw materials such as waste lipid or low-quality lipid. The possibility of using waste lipid derived from restaurant waste as non-edible lipid feedstock is gaining more attention due to the large amounts of restaurant waste generated at every day, and high amount of lipids contained within these wastes, up to 30 wt% [10]. Waste lipid is considered as a potential feedstock for biodiesel production because it is very low in value which will help to reduce biodiesel cost [11]. The potential of using restaurant waste as biodiesel feedstocks was reported [12]. However, this process generates a huge quantity of solid residual fraction which is consisted of starch, fat, protein and fibre after lipid extraction for biodiesel production [13]. It is possibility of the solid residual fraction harm



to environment without proper disposal. Thus, economically viable restaurant waste conversion technology must be developed and implemented.

Black soldier fly larvae, Hermetia illucens L. which can colonize a wide range of organic matters (animal manure, restaurant waste, and fermentation straw), can use the waste nutrition for development in which high protein and fat were synthesized [14,15]. Its life cycle is divided into four stages (egg, larva, pupa and adult) [16]. The adult flies are neither a nuisance species nor a mechanical vector of disease, as they do not need to feed, surviving on fat stores from their larval stage [17]. Mating takes place two days after emergence, and oviposition occurs two days after fertilization [18]. Larval development requires 2-4 weeks, depending on temperature and food availability [19]. The lipids derived from the energy insect larval which is converted from organic wastes such as the restaurant waste has been proved a novel and available feedstock for biodiesel [20-22]. It is reported that the larva contain on average 40-44% crude proteins and 35-40% lipids [23]. In contrast to the conventional higher plants or microalgae, the distinct advantages of the energy insect are fast growth rate, high biomass production, less growth time, and low land use. In addition, they can be grown in restaurant waste, poultry manure and can mitigate the problem of city pollution.

Multiple methods of lipid extraction from solid products have been tested such as Soxhlet extraction, mechanical pressing, solvent extraction, supercritical fluids extraction. However, these methods have many disadvantages such as long extraction times (Soxhlet extraction), low extraction yield (mechanical pressing), large amounts of solvent (solvent extraction), and high cost (supercritical extraction) [24–26]. Microwave irradiation is known to be very efficient as a biomass extractive-transesterification method. The cost of microwave heating is two thirds less than conventional heating and presents the potential of increasing lipids production rates from microalgae [27]. During this process, high heating rates are produced, effectively reducing processing times and solvent requirements [28]. Nevertheless, the application of microwave assisted for the lipid extraction from the energy insect has not yet been reported in literature.

Therefore, the aim of this work is to demonstrate the potential of microwave assistance as an eco-process for lipid extraction from the energy insect as a potentially energy saving system for the production of biodiesel. Influence of operating parameters on the extraction yield will be studied and evaluated. A response surface methodology obtained from a multivariate study was used to investigate the performance of the extraction procedure, to study the relevance of the variables required in extraction and to determinate the final optimal settings. In addition, GC, FTIR, DSC, and TGA were carried out to investigate the compositions and structural variation of lipids from the energy insect to understanding the mechanistic speculation of enhanced lipid yield of this insect species. Consequently, a direct feedback between modeling and experimental data in the present study should contribute to provide research bases for the analysis of kinetics and thermodynamics of lipid extracted from the energy insect in the next report.

2. Materials and methods

2.1. Sample preparation

The black soldier fly larvae (Fig. 1a) were obtained from the "Resources Utilization and New Energy Development" team in Wuhan Institute of Technology. The larvae were homogenized with homogenizer (Fig. 1b) and dried at 60 °C until constant weight was obtained.



Fig. 1. Black soldier fly larvae: (a) were colonized in the restaurant waste, (b) were homogenized with homogenizer, (c) crude larval lipid extracted.

2.2. Microwave-assisted solvent extraction experiments

Extractions were performed in a microwave system (Sineo Cor., MAS-II), having a maximum power output of 1.36 kW. The system consisted of 250 mL sealed Teflon holders with magnetic stirrers and a built-in optical fiber temperature sensor for process monitoring and control. 10 g dried insect powder was placed in holders with solvent (100 mL methanol, 100 mL acetone, 100 mL petroleum ether, 100 mL chloroform, 100 mL hexane, 60 mL hexane-40 mL methanol, 66 mL chloroform-34 mL methanol). This mixture was subjected to microwave treatment at different temperatures ranging from 30 to 60 °C for different extraction time. Crude larval lipid was then obtained by evaporating solvent with a rotary evaporator (Fig. 1c). The lipid yield was calculated using following expression:

Yield of bio-lipid (*wt.*%) =
$$\frac{\text{Weight of extracted lipid}}{\text{Weight of dried biomass powder}} \times 100\%$$

(1)

All of the experiments were performed in duplicate.

2.3. Experimental design

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Response surface methodology (RSM) was taken to obtain an optimal condition of the extraction method for the energy insect and to investigate the effect of critical variables including temperature (A), extraction time (B), and solute-solvent ratio (g/mL)(C) on extraction yield (Y). The experimental conditions for the RSM were temperatures of 30-60 °C, extraction times of 20-50 min, and solute-solvent ratio of 1:10-1:20 (g/mL). A total of 27 experimental runs of the three variables were designed by Box-Behnken design (BBD) using the Design-Expert software (Version 8.0, Stat-Ease, Inc., USA). A quadratic polynomial equation was used to fit the experimental data. Based on the response obtained, the statistical analysis such as analysis of variance (ANOVA), regression coefficient (R²) was executed. To evaluate the importance of extraction parameters, 3D plots were prepared. Finally, a validation experiment was conducted in triplicate and the correlation between the predicted and the experimental values were analyzed by correlation coefficient using Microsoft excel. Table 1 showed the variables and their levels used in the experimental design, and Table 2 represented the design matrix and the experimental results.

Table 1
The variables and their levels used in the experimental design.

Code	Variable	-1	0	1
А	Temperature (°C)	30	45	60
В	Time (min)	20	35	50
С	Solute-solvent ratio (g/mL)	1:10	1:15	1:20

Table 2

Box-Behnken experimental design by optimization of factors Time (min), Temperature (°C) and Solute-solvent ratio (g/mL) results of response of variables calculated in the form of extraction yield % (w/w).

Run	Temperature (°C)	Time (min)	Solute-solvent ratio (g/mL)	Yield (%)
1	60	50	15	32.68
2	30	20	15	27.56
3	45	35	15	28.14
4	45	50	10	30.12
5	45	35	15	28.07
6	60	35	10	29.88
7	45	50	20	30.79
8	60	20	15	28.87
9	45	20	20	27.74
10	30	35	10	28.78
11	45	35	15	28.16
12	30	35	20	28.46
13	45	35	15	28.24
14	45	35	15	28.11
15	60	35	20	29.83
16	30	50	15	31.05
17	45	20	10	26.95

2.4. Product analyses

2.4.1. Lipid transesterification and GC determination

Lipid obtained by microwave aided extraction was dissolved in methanol containing concentrated sulfuric acid as a catalyst (methanol-sulfuric acid volume ratio = 1:7). The mixture was then heated to 55 °C for 24 h. After the reaction, hexane was added the mixture and the mixed solution was stirred for 30 min at 20 °C. At last, NaCl solution was added (50 mL of 5% w/v NaCl solution per gram lipid) and the solution was allowed to stand for 15 min. Fatty acid methyl esters (FAMEs) were extracted in hexane phase (top). The bottom phase was again treated with hexane (40 mL per gram lipid, to remove non-recovered FAMEs) and FAMEs were separated and mixed with the fraction separated earlier. The FAMEs in hexane were washed with anhydrous sodium sulfate solution (20 mL of 2% w/v solution per gram lipid), and the top hexane layer was then dried at 50 °C in an oven. FAMEs composition of each sample were determined using an Agilent GC coupled with a mass spectrometric detector and a HP-5 column (length: 30 m; ID: 0.25 mm, phase thickness: 0.25 μ m). The column was ramped from 50 to 250 °C at 5 °C/min, and then held at 280 °C for 15 min. Nitrogen was used as the carrier gas with a flow rate of 45 mL/min.

2.4.2. Fourier transform infrared (FTIR) spectroscopy

FTIR can be utilized to identify some of the functional groups present in a solid, liquid or gaseous sample. In the present study, the functional groups of the lipid sample were analyzed by using a Nicolct 6700 FTIR Spectrometer (American Thermo Electron). The absorption frequency spectra were recorded and plotted as transmittance versus wave number.

2.4.3. Differential scanning calorimeter analysis (DSC)

The DSC profile of extracted lipid was obtained using DSC1 star^e system (Q2000, TA Instruments, USA) with aluminium crucible. Thermal program involved rapid heating of sample to 30 °C from room temperature (heating rate of 5 °C/min) and held under isothermal condition for 10 min. Then the extracted lipid was cooled from 30 °C to -50 °C.

2.4.4. Thermal-gravimetric analysis (TGA)

Thermal stability behavior of the extracted lipid was analyzed using TGA system (TGA-7, Perkin-Elmer, USA). Non-isothermal TGA experiment of lipid (~10 mg) was performed from ambient temperature to 700 $^{\circ}$ C at a heating rate of 10 $^{\circ}$ C/min. Nitrogen was used as carrier gas (flow rate of 30 mL/min).

3. Results and discussion

Solid-liquid extraction may be thought as a phase transfer of solute from one phase to another. The transfer mechanism is governed by capillary flow and depends upon solvent viscosity. Using microwave as the energy carrier causes direct generation of heat within the material, causing near instantaneous temperature rise within the matrix and pressure effects on the cell structure. The acceleration of extraction rates under microwave could be due to a synergy combination of the two transfer phenomena which of mass and heat acting in the same direction from the inside of the extracted material to the bulk solvent [29]. Therefore, a series of comparative experiments were conducted to clarify the impact mechanism.

3.1. Effect of process parameters: experimental study

3.1.1. The effect of the solvent types on the yield of extracted lipid

The extracted lipid yield was highly dependent on the solvent or mixture used [30]. Solvent mixtures containing a polar and a nonpolar solvent extracted a greater yield of lipid. In these cases, the polar solvent releases the lipid from their protein-lipid complexes, and the lipid subsequently dissolve in the non-polar solvent [31]. Depending on the extent of release by the polar solvent and the nature of the non-polar solvent, different extracted percentages are obtained [32]. The lipid extraction capabilities of different solvents (hexane, petroleum ether, chloroform, acetone, methanol, chloroform-methanol (C-M) = 2:1 and hexane-methanol (H-M = 3:2 for 50 min at 60 °C were shown in Fig. 2. As seen in Fig. 2, methanol resulted in a larger gravimetric yield than the other solvents. However, the recovered product was also found to contain a solid precipitate that was not present in the other samples. When the lipid phase was vacuum dried for measurement of gravimetric yield, the carryover material precipitated into a visible solid. Consequently, methanol was determined to "over extract" and give unrealistically high yield. The similar phenomena was observed in the previous report [33]. By comparing the yield of extracted lipid



Fig. 2. Effect of solvent types on lipid yield at the same extraction time of 50 min, solute-solvent ratio = 1:10 (g/mL), extraction temperature of 60 °C, and microwave power of 500 W.

from other solvents in the present study, it was found that the chloroform-methanol = 2:1 achieved the greatest lipid yield (34.06%) followed by chloroform (33.75%), hexane-methanol = 3:2(32.51%), hexane (31.78%) and petroleum ether (31.32%). Acetone alone as a lipid extraction solvent was demonstrated to have the lowest recoveries in the present study. The results indicated that the efficiency of the extraction process is a function of the molecular anity between solvent and solute in agreement with earlier studies [34]. The use of polar and nonpolar solvents combined with assisted energy has been reported to result in an emulsification-extraction process that results in the rapid and efficient extraction of total lipids from solid matrices [35]. The higher efficiency of the chloroform-methanol mixture has previously been reported [36]. Petroleum ether alone as a lipid extraction solvent gave yield slightly lower than the chloroform-methanol or hexanemethanol mixture in the present study. The reason has been attributed to the fact that nonpolar solvents only can extract neutral lipids like triacylglycerols [37,38]. However, polar lipids such as phospholipids can be lost [39]. Therefore, only the non-polar lipids are dissolved in the relative non-polar petroleum ether, explaining the lower percentage extracted when using this solvent. However, it should be mentioned here that the chloroform and hexane with high cost and toxic were used at the extraction process. Additionally, the most main disadvantage is that the polar compounds such as sugar and protein were dissolved in the methanol solvent with strong polar, which would introduced impurity and influenced quality of lipids at the extraction process. Therefore, the petroleum ether was chosen as the lipid extraction solvent at the last study because it is less toxic and the results of lipid extract obtained with this mixture was similar to the results obtained with chloroform:methanol (2:1, v/v).

3.1.2. The effect of microwave power on the yield of extracted lipid

The influence of microwave power on yield of lipid extraction at the solute-solvent ratio = 1:10 (g/mL) for 50 min was demonstrated in Fig. 3a. The enhancement of microwave power from 100 to 500 W resulted in the increase of extraction efficiency under the present conditions. The yield of lipid extracted at 200 W was 27.67%, higher than the value of 26.35% at 100 W. However, it was observed from Fig. 3a that the yield of extracted lipid remained approximately steady with a slight increasing tendency when the microwave power increased from 300 W to 500 W. For a solvent-



Fig. 3. Single factors effects of microwave power (A), extraction temperature (B), and solute-solvent ratio (g/mL) (C) on extraction lipid yield of the energy insect.

matrix to absorb microwaves, it has to preferably have a higher dielectric constant (potential for electric energy storage) as well as a higher dielectric loss (electric energy dissipation) [40]. Microwave radiation can cause efficient internal heating of the mixture by interacting with the sample matrix at molecular levels via dipolar rotation and ionic conduction [41]. At microwave frequencies dipoles in the sample align themselves with the direction of rapidly oscillating electric field and rotate, generating heat via frictional forces between randomly rotating polar molecules and surrounding media [42]. In ionic conduction, dissolved charged particles oscillate with the changing electric field, dissipating their kinetic energy via friction as they slow down and change direction. This friction in turn leads to localized superheating at higher microwave power [43]. Thus, under the higher microwave power irradiation the target compounds were immediately stimulated and subjected to high stresses, which cause promoting the release of the molecules of lipid.

3.1.3. The effect of extraction temperature on the yield of extracted lipid

In addition to the different solvent types, microwave power and time, the effect of extraction temperature was also studied. In Fig. 3b, there was variation in the yield of extracted lipid with increased in temperature from 20 to 60 °C. As presented in Fig. 3b, the crude lipid yield of about 26.8% was obtained when the temperature was 20 °C. Compared with the yield of extracted lipid at 20 °C, the higher yield of extracted lipid at 30 °C (about 28.95%). It can be seen clearly that the temperature has a great effect on the vield of extracted lipid. The maximum extraction vield obtained at 60 °C while increasing the temperature, lipid extraction yield proportionally increased to 31.32%. This behavior can be attributed to the enhanced mass transfer kinetics at higher temperatures. In general, the increase of temperature improves the extraction of lipid due to the weakening of hydrogen bonds between the intermolecular interactions with the solvent, which leads to better penetration of the solutes within the feedstock material move into solvent phase and diffuse out of the solid matrix faster at higher temperature [44,45]. In addition, solvent viscosity decreases at high temperatures and diffusivity increases; thus, also increasing extraction efficiency. Karlovic et al. investigated the effect of temperature on the kinetics of oil extraction from corn germ flakes prepared by a dry degermination process. Increase in the temperature can enhance the capacity of solvents to dissolve the oil because the thermal energy can overcome the cohesive and adhesive interactions [46].

3.1.4. Effect of the solute-solvent ratio (g/mL) on the yield of extracted lipid

Generally, a higher volume of solvent will increase the recovery in conventional solvent extraction techniques, but in microwave extraction a higher solvent volume may give lower recoveries [47]. This can happen depending on how the solute-solvent ratio is changed (maintaining the solute mass or the solvent volume constant), since this can influence the solution temperature and the recovery as previously shown. By comparison, a distinct difference can be observed for the system under study (Fig. 3c). For the trials at constant solvent volume, the ever-decreasing lipid yield when the solid powder mass was changed. The variation can be explained by the fact that the solid insect powder mass was gradually decreased. However, for the trials with constant insect powder mass, there was an apparent increase in lipids yield for solutesolvent ratio increasing from 10 to 20. It slightly decreased as the solute-solvent ratio higher than 16. This was probably due to the larger volume of petroleum ether causing excessive swelling of the material and absorbing the effective constituent [48]. Particularly, the liquid height in the beaker was lower than the microwave penetration depth for solvent at low solute/liquid ratio, which is reported to be 3.6 cm [49]. When the solvent volume was low, significant amounts of the electric field reach the back face of the sample to be reflected, so that the electric field in the sample is increased many folds, and the petroleum ether heating rate increases dramatically to more than ten times.

3.2. Optimization of extraction parameters by response surface methodology

In the present research work, the relationship between response yield of extracted lipid and three reaction variables such as effect of temperature, time and solute-solvent ratio were evaluated by using response surface methodology (RSM). Owning to the power settings of the microwave equipment in our laboratory, the influence of microwave power may not be considered in this section. The statistical software was employed to determine and evaluate the coefficients of the full regression model equation and their statistical significance. Regression analysis was employed to fit the empirical model with the generated response variable data. The quadratic polynomial equation was generated to predict the lipid yield using the design of experiment in the form of actual parameters (Eq. (2)).

$$Y = 36.8 - 0.43A - 0.13B + 0.03C + 3.56E^{-004}AB + 9E^{-004}AC - 4E^{-004}BC + 4.96E^{-003}A^2 + 3.46E^{-003}B^2 - 9.3E^{-004}C^2$$
(2)

where Y is the lipid yield, A is temperature ($^{\circ}$ C), B is time (min), and C is solute-solvent ratio (g/mL).

The result of statistical analysis of variance (ANOVA) which was carried out to determine the significance and fitness of the quadratic model as well as the effect of significant individual terms and their interaction on the selected responses were presented in Table 3.

When p-values was considered with regard to the each model, A, B, A², B² were significant model terms. But, the significant strength varies for the parameters. Among those parameters, B was the most significant as compared with other parameters (A, A², B²) because the p-values were less than 0.0001 for B. The low coefficient of variation (C.V.% = 1.02) indicated that the results of quadratic model was highly reliable. Statistically estimated R² value for fitted model determines the quality of the model. The R² value of 0.9829 and adjusted R² of 0.9609 showed that the model could be significant predicting the response and explaining 95% of the variability in the design. The statistical significance of the equation

Table 3				
Estimated	regression	coefficients	for	response

was evaluated by F-test and ANOVA (analysis of variance) which showed that the model was statistically significant at 95% confidence level (p < 0.0001). To investigate the interactive effects of operational parameters on lipid yield extracted, the threedimensional profiles of multiple non-linear regression models were depicted in Fig. 4, respectively, while the other parameter was kept constant.

The shape of the response surface curves showed a moderate interaction between these tested variables. The experiments for model validation were implemented under the slightly adjusted conditions. The lipid of extraction yield 32.68% was obtained under the adjusted condition microwave power 500 W, extraction time 50 min, extraction temperature 60 °C, solute-solvent ratio = 1:15 g/ mL, indicating good consistency with the predicted value 32.49%. The result of verifying experiment demonstrated good applicability of the established model.

3.3. Characterization of extracted lipid

3.3.1. Chemical compositions

Table 4 showed the chemical compositions of the larval lipid obtained under the predicting optimum extraction condition. One can see from Table 4 that 8 different fatty acid (FA) were detected and identified. The total FA contents in the raw extract were of 99.05% with the most abundant fatty acids being the oleinic and



Fig. 4. Response surface plots for the design conditions a) effect of extraction time and temperature, b) effect of solute-solvent ratio and time, c) effect of temperature and solute-liquid ratio.

Source	Sum of Squares	Degree of freedom	Mean square	F-Value	P-Value
Model	34.95	9	3.88	44.70	<0.0001
Α	3.66	1	3.66	42.11	0.0003
В	22.85	1	22.85	263.00	< 0.0001
С	0.15	1	0.15	1.71	0.2324
AB	0.026	1	0.026	0.29	0.6041
AC	0.018	1	0.018	0.21	0.6608
BC	3.600E-003	1	3600E-003	0.041	0.8445
A ²	5.25	1	5.25	60.44	0.0001
B2	2.56	1	2.56	29.43	0.0010
C2	2.276E-003	1	2.276E-003	0.026	0.8760
Residual	0.61	7	0.087		
Lack of Fit	0.59	3	0.20	48.97	0.0013
Pure Error	0.016	4	4.030E-003		
Cor Total	35.56	16			

Table 4

Comparison of fatty acids composition of lipid derived from the energy insect in present study and other biomass feedstock.

Fatty acid name	Energy insect	BSFL ^a	YMB ^b	Rapeseed ^c	SRF ^d	
Saturated fatty acids						
Capric acid (10:0)	1.13	3.1	1.2	-	1.8	
Lauric acid (12:0)	18.89	35.6	1.3	_	23.4	
Myristic acid (14:0)	9.91	7.6	8.1	_	3.7	
Palmitic acid (16:0)	20.96	14.8	17.6	3.49	18.2	
Stearic acid (18:0)	6.5	3.6	11.4	0.85	5.1	
Unsaturated fatty acids						
Palmitoleic acid (16:1)	6.45	3.8	9.3	-	9.4	
Linoleic acid (18:2)	12.67	2.1	16.3	22.30	5.8	
Oleic acid (18:1)	22.54	-	19.7	8.23	27.1	

^a The data was from Ref. [20].

^b The data was from Ref. [13].

^c The data was from Ref. [50].

^d The data was from Ref. [22].

palmitic acids (22.54% and 20.96%, respectively). Lauric acid was moderately abundant with about 18.89%. Compared to the other extracts obtained in YMB [20], BSFL [13], and rapessed [50], the lipid composition of the energy insect extract is different. Oleic acid was still the most abundant but with higher proportion than for the other extracts, however, there was very low proportion of palmitoleic acid (6.45%) in the energy insect extract compared to the other extracts. From the comparison, it can be found that the results obtained were almost similar to that reported by Zheng et al. [51].

3.3.2. FTIR analysis of the extracted lipid

Fig. 5 showed the FTIR spectra of the extracted lipid under the optimum extraction condition. As shown in Fig. 5, the strong absorbance between 2853.43 and 2923.30 cm⁻¹ corresponded to the aliphatic C–H stretching vibration, revealing a large amount of methyl and methylene groups [52]. The absorption peak near 1742.86 cm⁻¹ represented the C=O group stretching vibration in ketones or carboxylic acids, which was consistent with the high content of ketones in the lipid. The peaks corresponding to the presence of alkenes (-C=C- stretch) were found at 1574.38 cm⁻¹. The absorbance peaks between 1377.95 and 1464.56 cm⁻¹ revealed the X–H stretching vibration (X = C, N). These peaks confirmed the presence of aromatic amine (C–N stretch) was confirmed by the absorption band at 1165.75 cm⁻¹. The ester group (C–O stretch or



Fig. 5. FTIR spectra of extracted lipid under optimal prediction conditions.

C–H bend) was identified at 1095.45 cm^{-1} . Similar results were previously reported for the extraction of lipid from other lipid feedstock [53–55].

3.3.3. Cold flow properties

The obtained DSC profile for extracted lipid was shown in Fig. 6. Crystallization point of the sample was used to estimate the pour point on DSC curve. There were two exothermic peaks in the cooling program of DSC curve associated with the change in phase from liquid to solid. This revealed the presence of saturated and unsaturated fatty acids in extracted lipid from the energy insect [56]. The pour point of extracted lipid was found to be -12.90 °C. The pour point of the energy insect lipid is poor compared to canola oil (-18 °C) and castor (-22 °C) [57]. This trend is due to the presence of more saturated fatty acids in the present study (57.39%) compared to canola (7.37%) and castor oil (3.2%). The saturated fatty acids content in oil is responsible for its poor cold flow property; the more saturated fatty acid content poorer its cold flow properties [58,59]. Therefore, it is reasonable to deduce that the extracted lipid contents 57.39% saturated fatty acids (Table 4) in the present study which may be responsible for poor pour point value.

3.3.4. Thermal stability of the extracted lipid

The thermal characteristics of the extracted lipid were studied using a thermal gravimetric analyzer. The weight loss curves obtained by TGA and the derivative of the TGA curves (DTG) for the samples was shown in Fig. 7. According to Fig. 7b, The overall thermal decomposition of the extracted lipid could be divided into three stages, including dehydration between 30 and 160 °C, followed by devolatilization at 160-550 °C and solid residue decomposition between 550 and 700 °C. Similar results also been reported in other microalgae species [60,61]. In the dehydration stage, a weight loss was recorded in the extracted lipid at approximate 100 °C, which could possibly be attributed to the evaporation of water loosely bound to biomolecules [62]. Sudden decrease in weight of the lipid sample was started from 160 °C to 550 °C which may be due to the breakdown of heavier hydrocarbon molecules to lower molecular hydrocarbon, CO₂ and CO [59]. The lipid weight loss of 18% and 75% were obtained at 270 °C and 470 °C respectively (Fig. 7a). The maximum rates of weight losses for extracted lipid were occurred at temperature 385 °C. Finally, no residual weight was obtained after the degradation of the lipid.



Fig. 6. DSC profile of extracted lipid under optimal prediction conditions.



Fig. 7. TGA curve of extracted lipid under optimal prediction conditions.

4. Conclusion

The black soldier fly, Hermetia illucens, with its high content lipid is a novel and potential raw material for producing biodiesel. The insect lipid was extracted using a microwave assisted extraction technique. The effects of different process parameters such as types of solvent, microwave power, extraction temperature, extraction time, and solute-solvent ratio (g/mL) on lipid yield were investigated by practical experiment and model design experiment. The effects of extraction time, extraction temperature, and solutesolvent ratio (g/mL) were found to be significant. An optimum vield of 32.49% on weight basis was obtained at conditions 15 g/mL of solute to solvent ratio, 50 min extractions time, and 60 °C extractions temperature. The fatty acid compositions of the extracted lipid were estimated by GC-MS technique was found to be 22.54% oleic, 12.67% linoleic, and 6.45% palmitoleic acid. The physicochemical characteristic of lipid revealed that it can be considered as a potential source for biodiesel production. Therefore, it is well conceived that extraction lipid of black soldier fly using the waste nutrition (animal manure, the restaurant waste, and fermentation straw) provides a novel and promising option for large-scale production of biodiesel.

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