Journal of Sustainability Science and Management
Volume 17 Number 12, December 2022: 49-66
eISSN: 2672-7226

STUDY ON THE EXTRACTION OF BLACK SOLDIER FLY (HERMETIA ILLUCENS) LARVAE OIL BY USING MECHANICAL PRESS METHOD

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Submitted final draft: 29 June 2022 *Accepted: 4 August 2022* http://doi.org/10.46754/jssm.2022.12.006

Abstract: This study investigates the optimisation of Black Soldier Fly oil extraction from different feeding sources. The larvae are fed a mixture of pasta-tobacco waste (MPT) and fish-ball sludge (FS). The mechanical press method is used to extract the oil. The results showed that the MPT-fed larvae had a higher weight at 0.088 ±0.001 g compared to 0.062 ± 0.002 g for larvae fed with FS. The feeding rates revealed that the higher the dose of the feed, the more weight the larvae gained. Meanwhile, GC-MS results show that the MPT substrate has a higher number of characterised fatty acids than the FS substrate, with three predominant fatty acids being lauric acid (43.69%), palmitic acid (16.15%) and linoleic acid (12.35%). Fourier-Transform Infrared spectroscopy (FTIR) analysis for both substrates reveals a similar high absorbance but High-Performance Liquid Chromatography (HPLC) analysis shows glutamic acid to be the most abundant amino acid in both substrates. Additionally, the regression analysis shows that moisture content and feeding rate is the most important parameter. Response Surface Methodology (RSM) determined that moisture content and feeding rates were the best parameters for oil yield.

Keywords: Black Soldier Fly (BSF), extraction, mechanical press method.

Introduction

The Sustainable Development Goals (SDGs) aim to ensure sustainable resource management and utilisation by 2030. Malaysia has reiterated its commitment to long-term sustainability and the environment to maintain a sustainable environment. This corresponds directly to the Twelfth Malaysia Plan (12MP) which focuses on shared prosperity initiative and consists of three pillars that echo the sustainability trifecta: Economic empowerment, environmental sustainability and social re-engineering. Within this plan, the environmental sustainability pillar highlights concepts such as green technology, renewable energy, mitigation and blue economy. The development of the environment has caused a crisis in solid waste management, especially in the industrial sector that generates organic waste. Therefore, Malaysia has proposed a 5R strategy to increase public awareness, which builds from the current 3R (Reduce, Reuse, Recycle) by adding the Refuse and Remove elements. The use of this term in a circular economy indicates a tendency to manage, reduce or improve the economy of waste resources which are used in 5R applications.

In a previous study, Smetana *et al.* (2019) mentioned a new insect production application that can be related to the 5R concept. Insects are versatile creatures that can be used for animal feed and are safe for human consumption. The use of insects as waste-reduction agents in the industrial sector is still scarce. The management of waste, especially from the manufacturing sector, is difficult to maintain sustainably and is still being disposed of in landfills. As Chia *et al.* (2018) mentioned, one approach for an ecologically friendly and cost-effective innovation is to cultivate insects such as the black soldier fly (BSF) within organic waste streams, as the organic content of the waste provides a source of nutrition for the BSFs.

Previous studies established that BSF can efficiently dispose of organic and inorganic waste by converting it to protein- and fat-rich biomass. Furthermore, BSF is not a pest or a carrier of human disease (Ravi *et al.,* 2020). BSF larvae fed with other types of waste typically have high levels of crude protein (above 33%) (Zhao *et al.,* 2017; Hasnol *et al.,* 2020). The amino acid profile is very similar to that of soybean meal (SBM) with the same protein content (44%) (Zhao *et al.,* 2017). Hasnol *et al.* (2020) also discovered that the amino acid profile of BSF larvae exceeds the Food and Agriculture Organization (FAO) requirements for SBM and sunflower and that the methionine level exceeds the FAO's fishmeal standard. As a result, BSF is regarded as a promising long-term approach to organic waste management, largely due to its bioremediation potential (Gikuru & Moriasi, 2007; Nde & Foncha, 2020).

Solvent extraction and mechanical pressing are two common methods for extracting BSF oil. The concentration of fatty acids in BSF oil extracted by the mechanical press is comparable to oil extracted by solvent extraction, both of which have advantages and disadvantages. Solvent extraction is a costly method that results in foam deposits during distillation. Meanwhile, mechanical presses have a low initial investment and require little labour (Santoso & Inggrid, 2014). It is also used on a small scale, making it ideal for extracting oil in less developed areas. The product has no organic solvent and is free of chemical contaminants (Zhao *et al., 2*017). The desire to obtain high-quality crude oil, ecological conservation and the adaptability of the system to continuous processes are the reasons for the use of mechanical systems (Wang *et al.,* 2017). Given the insufficiency of research on insect oil extraction via mechanical press methods. As a result of its effectiveness in extracting high-quality oils from various sources such as crop seeds and legumes as stated in previous studies, this method is highly recommended by Gikuru and Moriasi (2007). A recent study by Debnath *et al.* (2014) has also shown that this technology is convenient and environmentally friendly.

In order to maximise oil yield, various other factors or conditions can be manipulated during extraction. These factors, which include the moisture content of the sample and feeding rates, all directly impact the yield. However, control over these two factors may be limited in some types of extractors due to design and operation requirements (Santoso & Inggrid, 2014; Hasnol *et al.,* 2020). Following that, using various mathematical and statistical techniques, this study models and analyses the problem in two or more process variables that affect the intended response. Response Surface Methodology (RSM) reduces the number of experimental runs while maintaining expected accuracy and determining responses to multiple variable interactions. Among the RSM designs available, the Central Composite Design (CCD) and the Box-Behnken Design (BBD) have been widely used for oil extraction processes (Nde & Foncha, 2020).

Therefore, the goals of this study are: (i) To investigate the optimisation of BSF oil extraction from different wastes, (ii) To analyse the presence of functional groups in extracting oil and identify amino acids in extracting oil protein and (iii) To establish a model and simulate the extraction process using Response Surface Methodology (RSM).

Materials and Methods

Black Soldier Fly Larvae (BSFL) Rearing

The rearing of BSF larvae was carried out in a suitable environment for larvae growth, with ambient temperature and pressure. The egg of BSF was purchased from the company Unique Biotech Sdn. Bhd. Two containers were prepared with different substrates (Figure 1); one with mixed pasta-tobacco (MPT) and another one with fish-ball sludge (FS) to house the BSF egg. 500 g of the egg was then placed in each prepared container. The tobacco and fish-ball sludge are provided by the Phillip

Morris tobacco factory and Fusipim Company in Sungai Besar, Selangor while the pasta is an expired product obtained at a nearby store in Shah Alam. It took 12 days for the BSF egg to transform into larvae. The growth was tracked daily until the egg fully transformed into larvae on day 12. By then, the BSF larvae had formed and were ready to be tested for another phase: Larvae feeding and sampling between days 13 and 16.

Larvae Feeding and Sampling

The larvae were divided and fed at ranges of 50, 100, 150 and 200 mg/larvae/d according to the studied procedure (feeding speed and feeding method), atmospheric temperature (28 ± 2.0°C), relative humidity (65 ± 5%) and the moisture content of the raw materials under these conditions (Nyakeri *et al.,* 2019). For every study, samples were collected randomly from each container on day 12 by extracting 100 larvae randomly. The sampled larvae are weighed collectively on an electronic scale before being returned to another 4 breeding containers. This is followed by the calculation of the average weight of each larva by dividing the total weight by the number of larvae. The weight gain was calculated for the last 4 days, from day 13 to day 16 by comparing the obtained average larval weight to the previous average. The larvae and any remaining larvae were harvested separately on the last day of the experiment, supplemented by manual picking. The total weight of the biomass and residue was then weighted. Before the drying process in the oven was started, all larvae were killed with hot water. The larvae were then used for further experiments, drying in an oven to obtain the moisture content.

Moisture Content Analysis

The amount of water in a sample is defined as its moisture content. Specific procedures were followed to prepare samples of BSF larvae with moisture content following standard laboratory procedures (AOAC, 2000). The moisture content is obtained by drying the sample to a constant weight in an oven. Care was taken to ensure an equal drying rate for each sample to ensure no larval samples overlapped. First, the tray was dried in the oven and weighed using an electronic scale. A black soldier fly larvae sample was placed on the weighing tray and weighed together. The dried samples were placed in an oven at 45°C. Every 30 minutes, the samples in the sample tray were removed from the oven and weighed to determine the moisture content. The moisture content of the sample can be calculated using the formula below. The drying process will be terminated

(a) (b)

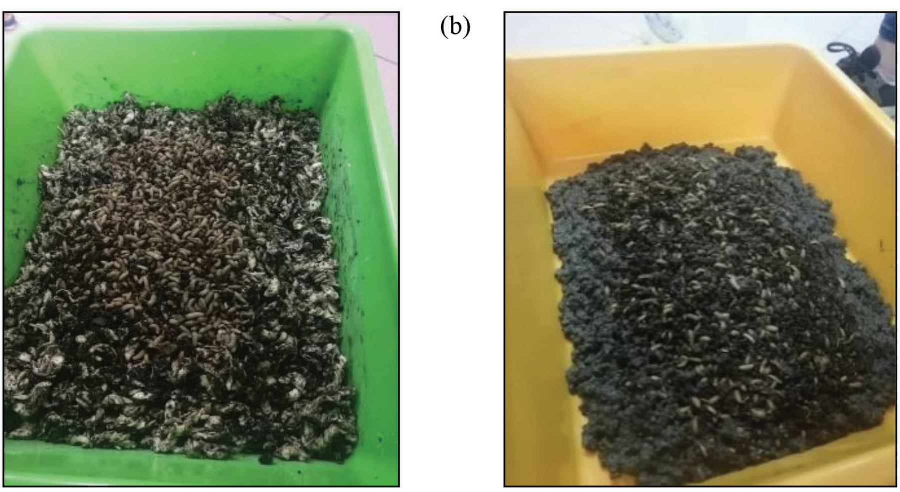

Figure 1: Both containers filled with (a) MPT, (b) FS

once the calculated moisture content falls below 10%. The percentage of moisture content of the dried sample was calculated using Equation 1.

$$MC(\%) = 100 - \left(\frac{m_1 - m_2}{m_1 - m_0} x100\right) \quad (1)$$

where m_0 = mass of empty tray (g), m_1 = mass of tray with sample before sample drying (g) and m_2 = mass of tray with sample after sample drying (g).

Extraction of BSFL Oil by Using the Mechanical Press Method

The mechanical-press extraction of BSF oil commenced when the best moisture content was determined. The BSFL oil was extracted using a squeezer tool. Each extraction process requires 50 g of sample. The extraction rate was calculated before and after the pressing process. The following study will use the oil yield and moisture content results to determine the best variables for extracting the most BSFL oil. The extraction of BSFL oil was studied by calculating using Equation 2.

$$\text{Oil yield } (\%) = \frac{\text{mass of oil collected (g)}}{\text{initial mass of sample (g)}} x100 \quad (2)$$

Compound Identification by Using Fourier Transform Infrared (FTIR)

FTIR spectra were analysed using KBr discs to detect functional groups (El Mansouri & Salvadó, 2007). The purified polysaccharide fraction was ground with 300 mg of KBr powder and then pressed into granules to convert the spectral IR of the Mattson instrument from 550 to 4,000 cm-1. The white spectrum of a temperature-calibrated tungsten strip lamp has been corrected for the efficiency of wave-related signal detection in the setup. Before starting the FTIR analysis, attenuated total reflection, Nujol or other techniques were used to prepare samples for testing. Adequate samples are required to acquire the absorption spectra. The FTIR spectrometer generates a chart of an absorption spectrum, showing the sample material's unique chemical bond and molecular structure. There will be peaks representing the components present in the absorption spectrum. These absorption peaks indicate functional groups (such as alkanes, ketones and acid chlorides). Different kinds of bonds and functional groups absorb various wavelengths of infrared radiation (Amir *et al*., 2013). Then in the reference library program, the classification spectrum of the known material was used to compare and analyse the spectrum to identify the composition or find the "best match" of the unknown material.

Characterisation of Compounds by GC-MS

The extracted BSF oil was diluted with 2 mL hexane solvent in a one-to-one ratio (Ravi *et al.,* 2019). Spectrum Laboratories used Agilent Technologies 7890A/5975C Series MSD apparatus to characterise BSFL oil compound by GC-MS. This device includes a non-polar Agilent HP-5MS column with a length of 30 m, a diameter of 0.25 mm and a thickness of 0.25 m according to the method by Chen *et al.* (2012). The analytical conditions were as follows: Sample injection (1 L), carrier gas, helium (99.999% pure) and flow rate 1 mL/min with split mode on, ratio 50:1, injector temperature 250°C and split the flow of 50 mL/min. The oven temperature was set to 60°C for 10 minutes before being increased at a rate of 3°C/minutes to 230°C which had to be maintained for 1 minute. The compounds identified were based on the National Institute of Standards and Technology's mass spectral library (NIST).

Amino Acid Composition in Protein of Extracted Oil by Using HPLC

An Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, self-sampler, column heater and variable wavelength detector (VWD) was used to analysed the extracted product. The column used is Agilent ZORBAX Eclipse XDB C18 (4.6 x 150 mm, 3.5 m), with the temperature set at 25°C. Mobile phase A is methanol and mobile phase B is water + 0.1% TFA (trifluoroacetic acid). The flow rate through the column is 1 ml/minute. The gradient is

90% B at 0 minute and 65% B at 18 minutes. The injection volume is 10 L according to the method specified by Fan *et al.* (2006).

Statistical Analysis of Percentage Yield of Extracted Oil by Linear Regression

There are two types of linear regression: Simple linear and multiple linear. Linear regression is used to create a regression model using the data from the adsorption experiment. Using the oil factor to select independent and dependent variables in a linear model, the dependent variable was chosen to be the percentage of oil yield. Meanwhile, the independent variables were chosen as moisture content, extraction time and feeding rates. Equation 3 represents the general equation proposed for regression modelling.

$$Y_n=aX_n+bX_n^2+c \quad (3)$$

Y_n = Oil yield percentage (%), X_n = moisture content, extraction time and feeding rates.

Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a well-known analytical tool in optimisation. RSM's function is to design the experiments, build the research model, estimate the effect of the parameters and investigate the optimum conditions to achieve the desired response (Ishak *et al.,* 2018; Su *et al.,* 2019). Furthermore, RSM can optimise multiple variables simultaneously, reducing the number of experimental runs (Su *et al.,* 2019; Nde & Foncha, 2020).

Summary of Research Flow

Figure 2 shows the holistic methodology illustration, all of the research methodologies involved were integrated phase by phase to achieve the objectives stated. Overall, there are three phases involved: Preparation, experimental and analysis.

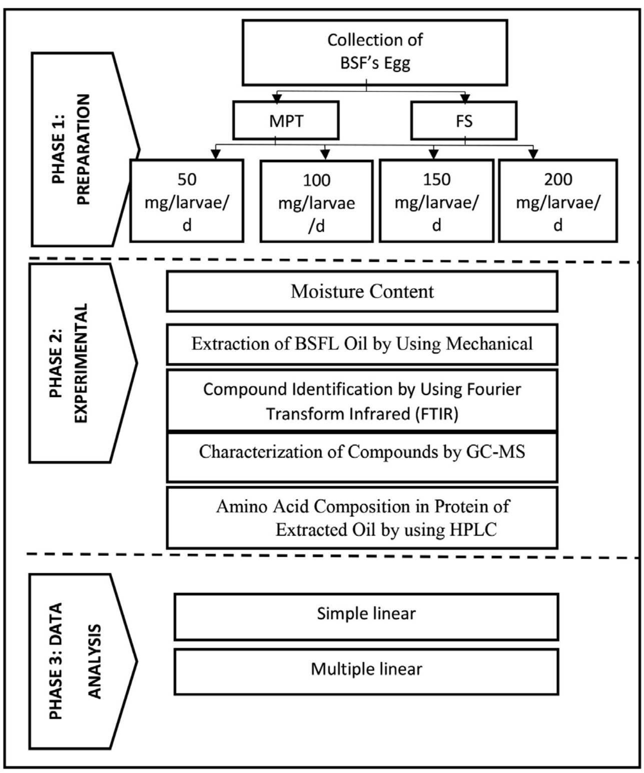

Figure 2: Research flow

Results and Discussion

Effect of Feeding Substrates on Larvae Growth

There are two feed sources used in this study: Mixed Pasta-Tobacco (MPT) and Fish-ball Sludge (FS). Based on Table 1, MPT produced considerably higher average larvae weights of 0.088 ± 001 g at the same feeding rate of 100 mg/larva/day while FS produced the lowest average larvae weights (0.062 ± 002 g). Similar results were observed for total larvae yield, with MPT yielding much higher larvae (187.9 ± 5.0 g) than FS (128.5 ± 4.0 g). Figure 3 presents the weight gain of larvae from different substrates during the different stages of development. Figure 3 depicts the weight increase of larvae in 12 days for both substrates. Larvae-fed MPT waste gained more weight than FS-fed larvae. The lower weight in FS could be because the waste is no longer fresh by the time it is used to feed the larvae as the sludge colour turns black, as opposed to the orange colour of fresh sludge. The term "blackish sludge" refers to old sludge that has been stored for a long time.

Optimisation of the Production of BSFL

Effect of Feeding Rates on the Growth and Weight of Larvae

As shown in Figure 4, feed rate influenced the growth rate of larvae fed with the major homogeneous substrate, Mixed Pasta-Tobacco (MPT), starting from day 13 till 16. Observations indicated that higher feeding rates result in faster growth, while lower feeding rates contribute to lower growth rates. On day 13, the performance of 50 mg/l/d was significantly lower than other feed rates. The performance of 50 mg/l/d and 100 mg/l/d was significantly lower than that of 150 mg/l/d and 200 mg/l/d from day 13 to day 16.

Different feeding rates were used instead of the standard feeding rate recommended by Lalander *et al.* (2019) for the other substrates (FS) and this resulted in a significant increase in both larvae weight and yield. This was observed for the homogeneous substrates used in the study, which includes the control treatment. The feeding rate was increased from 50 to 200 mg/larva/day, which increased larvae weight and yield (Table 2). The amount of food available

Table 1: Summary weight and yield of BSF larvae on two feed sources

Feedstock	Larvae Weight (g)	Larvae Yield (g)
Fish-ball Sludge (FS)	0.062 ± 0.002	128.5 ± 4.0
Mixed Pasta-Tobacco waste (MPT)	0.088 ± 0.001	187.9 ± 5.0

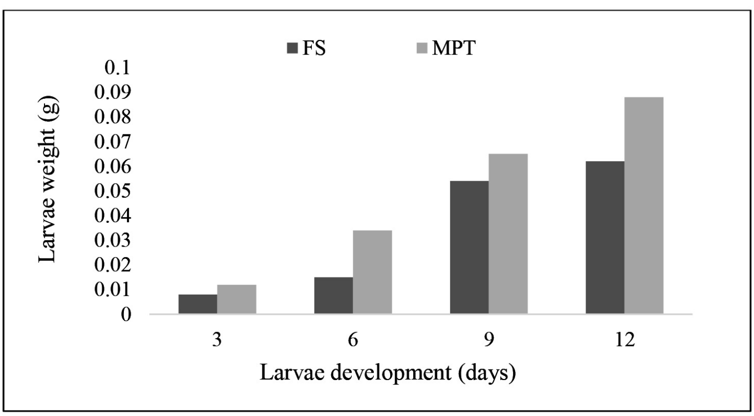

Figure 3: The different weights of BSF larvae during growth development in different feedstock

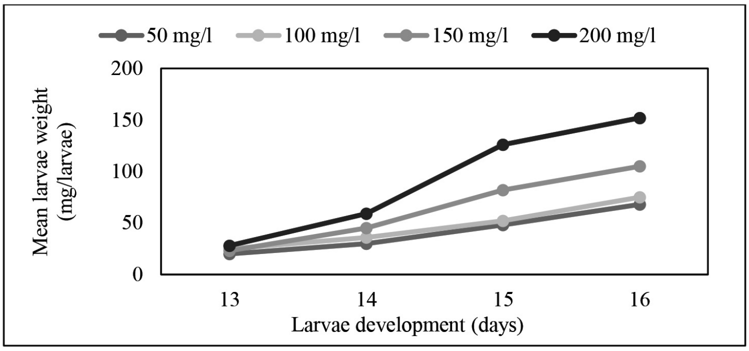

Figure 4: The effect of feeding rates on the growth of BSF fed on a mixed pasta-tobacco waste

Table 2: Effect of different substrate feeding rates on larvae weight and larvae yield (mean ± standard deviation)

Substrate	Feeding Rate (mg/l/day)			
	50	100	150	200
FS				
Mean larvae weight (g)	0.038 ± 0.002	0.085 ± 0.007	0.092 ± 0.002	0.105 ± 0.004
Larvae yield (g)	80.5 ± 2.0	128.5 ± 4.0	205.8 ± 12.0	228.5 ± 6.0
MPT				
Mean larvae weight (g)	0.042 ± 0.004	0.082 ± 0.005	0.097 ± 0.001	0.112 ± 0.005
Larvae yield (g)	92.4 ± 6.0	135.2 ± 12.0	212.4 ± 4.0	238 ± 6.0

also influenced the time it took for the larvae to mature. For instance, at 150 mg/l/d and 200 mg/l/d, a significant portion of the larvae population turned into prepupae by the 15th and 16th days, respectively, much faster than those fed 50 mg/l/d and 100 mg/l/d, where prepupae stage started on the 18th and 20th days.

Effect of Moisture Content

Moisture content is one of the important elements in this study. The moisture content of the sample is important because it can affect the extraction rate of oil when using the mechanical – press technique and indirectly affects the percentage of extracting oil yielded by the technique (Lalander *et al.,* 2019). In this study, the moisture content of BSF larvae was reduced to <10% to increase the extraction efficiency of the larvae. According to Equation 1, the BSF larvae were dried to a moisture content of 8.25% for Mixed Pasta-Tobacco (MPT) and 9.99% for Fish-ball Sludge (FS). According to Ishak *et al.* (2018), the highest oil yield was obtained at a moisture content of 9.3%, resulting in 97% biodiesel from BSF larvae.

In addition, Deli *et al.* (2011) also indicated that the moisture content of larval oil suitable for mechanical pressing is normally between 7-8%. The lower the moisture content, the higher the oil yield from the extracted larvae. This demonstrated that reducing the moisture content of a sample during the extraction process results in a higher percentage of extraction yield. As in this study, the moisture content of dried larvae was 8.25% for MPT and 9.99% for FS.

Effect of Moisture Content on Pressing Time to Extract BSF Oil

The best pressing time for BSF larvae oil was determined using the mechanical-press method during ambient temperature and pressure. The extraction process lasted 150 minutes, with the extracted oil being collected, weighed and

Table 3: The data of moisture content of each substrate versus time

Time (minutes)	Moisture Content of Different Substrates (%)	
	FS	MPT
30	90.01	88.98
60	70.55	71.27
90	39.05	48.78
120	19.21	21.6
150	9.99	8.25

recorded every 30 minutes. Figure 5 verifies the best drying time of BSF larvae oil. The graph of moisture content versus drying time for both substrates is shown in Figure 5 (a). The lower the percentage of moisture content in larvae, the longer the drying time. At 150 minutes, the moisture content in MPT-fed larvae waste is slightly lower than that of FS-fed larvae, with 8.25% and 9.99%, respectively. Meanwhile, Figure 5 (b) shows that the highest percentage of extracted oil is at 60 and 90 minutes, with 92.61% and 97.14%, respectively. The graph also shows the pattern of the drying rate slightly decreasing due to the diffusion-controlled at 120 minutes for MPT. However, the percentage oil yield for FS is not as high as MPT. As a result, the ideal drying period for BSF larvae oil was 60 to 90 minutes, depending on the number of BSF larvae used. This coincides with a previous study conducted by Ishak *et al.* (2018) that indicates the lack of need to continue the extraction process because the extraction rate is nearly in a steady state.

Identification of Compounds in Extract BSFL Oil

FTIR Analysis of the Extracted Oil

Figure 6 (a) depicts the isolated lipid's FTIR spectra under optimal extraction conditions. The strong absorbance between 2,866 and 2,941 cm^{-1} matched the aliphatic C-H stretching vibration while Figure 6 (b) also shows a similar strong absorbance between 2,838 and 2,952 cm^{-1}. It suggests a substantial number of methyl and methylene groups [17] as illustrated in Figure 6. At 1,596 cm^{-1}, the peaks related to the presence of alkenes (-C=C- stretch) were discovered. The absorbance peaks indicated the X-H stretching vibration (X=C, N) at 1,455 cm^{-1} based on Figure 6 (b). These peaks confirmed the presence of triglyceride functional groups in the energy bug. At 1,017 cm^{-1}, the ester group (C-O stretch or C-H bend) was detected. Previously, similar results were reported for the extraction of lipids from different lipid feedstock (Dutta, Sarkar & Mukherjee, 2014; Reshad, Tiwari & Goud, 2015).

(a) (b)

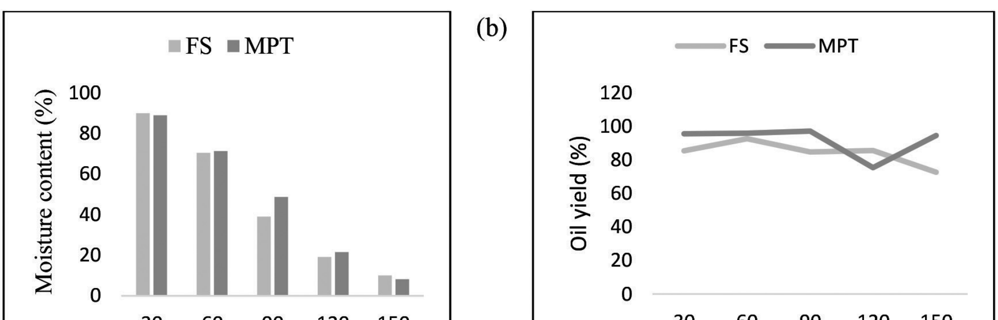

Figure 5: The graph of drying time versus (a) moisture content, (b) oil yield for both substrates

Nutrient Composition of Fatty Acids in BSF Oil Extract Using GC-MS

Figure 7 shows the findings of GC-MS analysis of BSF fatty acid components. Figure 7 (a) of MPT substrate shows the first peak representing capric acid (C10:0) appeared at 18 minutes while the other major peaks representing myristic acid (C14:0) (11.88%) appeared at 48 minutes, with palmitic acid (C16:0) (16.15%) appearing at 57 minutes, oleic acid (C18:1) (14.3%) at 71 minutes and linoleic acid (C18:2) (12.35%) at 170 minutes. The other fatty acid composition of MPT is present in Table 4. As a result of the component of fatty acids in the FS substrate, data indicates that a few types of fatty acid appeared in the chromatogram [Figure 7 (b)]. From Figure 7 (b), the first peak is lauric acid (C12:0), which appeared at 19 minutes, followed by myristic acid (C14:0) (12.81%) at 48 minutes and palmitic acid (C16:0) at 74 minutes. Meanwhile at 95, 109 and 135 minutes, compositions of fatty acid or omega 6 fatty acids, butyric acid (C4:0) and omega 3 fatty acids appeared, respectively. Both substrates had a distinct fatty acid composition, with the MPT substrate having a higher amount of characterising fatty acids than the FS substrate in the chromatogram. According to Saraswathi and Hameed (2016), the predominant fatty acids found in the pasta samples were palmitic, palmitoleic and linoleic. This demonstrates that the primary fatty acid found in MPT is consistent with earlier investigations. While the fatty acid content of FS is reduced, this could be due to the nutrients lost during storage.

Composition of Amino Acid in Protein of Extracted BSFL Oil Using HPLC

Table 5 illustrates the findings of HPLC analysis on the amino acid content of BSF protein for both substrates. Glutamic acid was the most abundant amino acid in MPT and FS protein, with 17.4% and 15.3%, respectively. According to Janssen *et al.* (2017) and Müller, Wolf and Gutzeit (2017), glutamic acid was the most common amino acid in BSF. Similarly, glutamic acid is the most frequent amino acid component in the prepupa stage of the BSF sample (Surendra *et al.,* 2017). Protein from other insect species such as tropical banded crickets (Gryllodes sigillatus), contains 19% glutamic acid, making it the most dominant amino acid (Hall *et al.,* 2017).

In this study, protein from BSF has a comparatively high amount of alanine (11.2% for MPT and 9.8% for FS). This is because the bromelain enzyme employed in the BSFL hydrolysis process was primarily composed of the amino acid L-alanine (Murachi, 1964). BSFL protein includes nearly every necessary amino acid. The most abundant essential amino acids in MPT and FS were hydrophobic amino acids such as lysine (8.5% and 7.2%), leucine (8% and 8.7%) and valine (4.4% and 5.7%). A high concentration of hydrophobic amino acids in a protein can indicate its ability to scavenge or block free radicals.

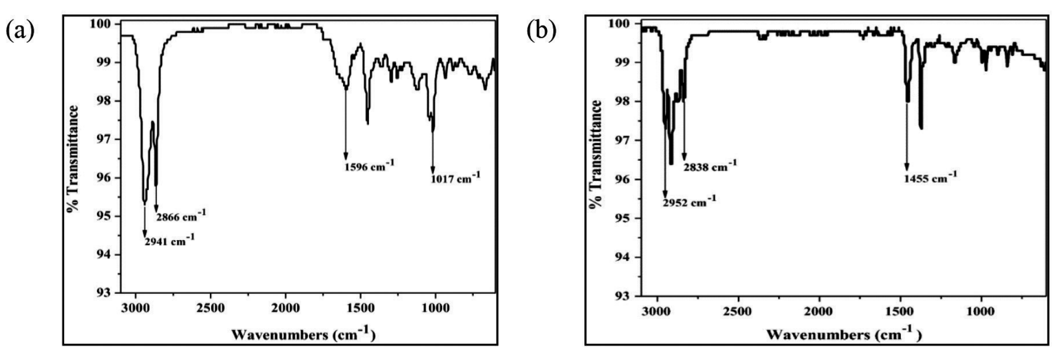

Figure 6: FTIR spectra of extracted lipid under the highest predictive conditions for both (a) MPT and (b) FS substrates

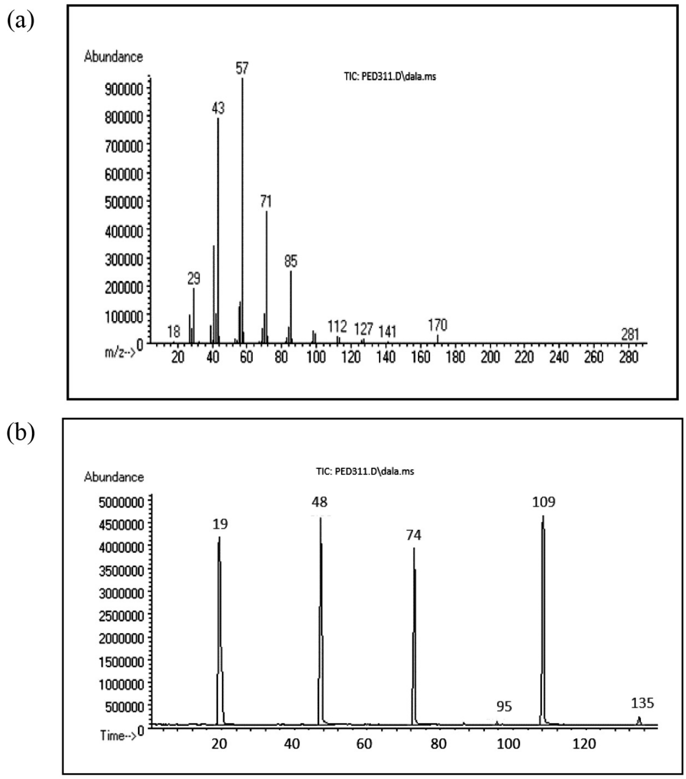

Figure 7: Chromatogram of BSFL oil extracted that feed on (a) Mixed Pasta-Tobacco (MPT), (b) Fish-ball Sludge (FS)

Table 4: Composition of fatty acid in BSF larvae oil

Identified Fatty Acid	Retention Time (minutes)	FS	MPT
C12:0 (Lauric acid), %	19.0	37.52	43.69
C10:0 (Capric acid), %	18.0	-	8.43
C14:0 (Myristic acid), %	48.0	12.81	11.88
C16:0 (Palmitic acid), %	57.0	9.76	16.15
Omega 6 fatty acids, %	95.0	1.78	2.65
C16:1 (Palmitoleic acid), %	141.0	-	1.63
C18:0 (Stearic acid), %	29.0	-	0.52
C18:1 (Oleinic acid), %	71.0	-	14.3
C18:2 (Linoleic acid), %	170.0	-	12.35
Omega 3 fatty acids, %	135.0	0.76	0.54
C14:1 (Miristoleic acid), %	85.0	-	0.13
C17:0 (Heptadecanoic acid), %	112	-	0.01
C4:0 (Butyric acid), %	109	0.22	0.02

Table 5: The comparison of amino acid composition in the protein of extracted oil for MPT and FS with the previous study

Amino Acids	This Study (Protein from BSF with Different Substrates)		Protein from *G. sigillatus* (a)	BSF (b)	BSF (c)	BSFL Prepupae (d)
	MPT	FS				
Alanine	11.2	9.8	16.6	4.7	6.2	7.9
Arginine*	6.2	6.6	18.1	4.6	6.2	6.6
Aspartic acid	10.8	9.2	0.8	12.6	10.3	7.8
Cysteine	0.2	2.4	3.6	1.33	0.5	3.3
Glutamic acid	17.4	15.3	19.9	12.1	12.2	8.4
Glycine	5.3	3	3.7	3.9	5.4	7.3
Histidine*	4.7	3.8	3.4	3.6	4.8	5
Isoleucine*	5.8	3.4	2.6	5.8	4.8	4.5
Lysine*	8.5	7.2	3.6	9.2	7.4	6.5
Leucine*	8	8.7	8.4	8	7.7	6.9
Methionine*	1.1	0.2	0.6	2.5	0.6	2.6
Proline	5.4	3.5	3.3	4.34	6.2	6.2
Phenylalanine*	6.4	4	2.6	7.2	6.2	4.4
Serine	4	3.9	6.7	4	4.1	4.5
Tyrosine	0.4	1.5	1.4	6.3	6	7
Threonine*	4.7	4.9	2.6	4.9	4.5	4.4
Tryptophan*	0	0	0.7	0	0	0
Valine*	4.4	5.7	1.4	5.6	6.7	7.2

Note: (a) Hall *et al.,* 2016, (b) Janssen *et al.,* 2017, (c) Muller *et al.,* 2017, (d) Surendra *et al.,* 2016

Linear Regression Analysis on the Percentage Yield of Extracted BSF Oil

Table 6 displays the results of regression statistics of the percentage yield of BSF oil for moisture content, extraction time and feeding rates. The adjusted R^2 for the multiple regression of the percentage oil yield for moisture content and feeding rates was 0.98386 and 0.97344, respectively. Simple linear regression was used for the extraction time. As a result, the R^2 value was only 0.00632. Based on the results, the data for variable moisture content and feeding rates were fitted away because the values were close to 1 but only the data for extraction time was poorly fitted. The predictor variables were correlated to the percentage of oil yield by 98.386%, 97.344% and 0.632%, respectively in all R^2 values. As a result, it is possible to conclude that the coefficient determination for percentage oil yield has a stronger relationship between the independent and dependent variables, as most of the variables in this study were found to be close to 1 for adjusted R^2 values.

Table 7 depicts the coefficients model and the coefficient for the percentage yield of BSF oil in each variable. A significant coefficient's dependability can be determined using the P-value. The probability of the regression output being obtained by chance or the probability of the rejection of the null hypothesis is higher if the P-value is smaller than 0.05. A P-value greater than 0.05, on the other hand, suggests that the regression result was achieved by chance or by the null hypothesis. It can also be

Table 6: Regression statistic for percentage yield of extracted BSFL oil for each of the variables

Regression Statistics			
Variables	**Percentage of Oil Yield**		
	Multiple R	**R Square**	**Adjusted R Square**
Moisture content	0.991897235	0.983860124	0.978480166
Extraction time	0.079497244	0.006319812	-0.324906917
Feeding rates	0.986629811	0.973438383	0.964584511

described as insignificant. The regression for the percentage yield of BSF oil in terms of moisture content and feeding rates was less than 0.05 but it exceeded 0.05 in extraction time. Some of the output may be considered significant but others may be marginally significant. As a result, the regression output can be calculated using the mathematical model equation. Table 7 was used to construct the equations. Using unstandardised coefficients data, the multiple linear regression modelling for the percentage of oil yield is listed in Equations 4 to 6.

$$Y_1 = -0.339X_1 + 96.98 \quad (4)$$

$$Y_1 = -0.001X_1 + 95.54 \quad (5)$$

$$Y_1 = -0.009X_1 + 96.46 \quad (6)$$

The most important factors in BSF oil percentage yield were moisture content and feeding rates. According to the statistical results, the models in Equations 4 to 6 were suitable for predicting the percentage yield of BSF oil within the selected experimental range variables. To observe the performance model, the plotted graph for actual and predicted versus experimental percentage yield of oil was shown in Figure 8. As a result, the predicted values for the percentage oil yield for each variable were almost similar to their experimental values because their predicted R^2 values were greater than 0.05 and closer to 1. Furthermore, a percentage error of less than 1% indicates that the value is close to the experimental values. Because the evaluated percentage error was less than 1%, it is reasonable to conclude that all the regression models fit well in predicting the percentage of oil yield.

As a result, moisture content is the most important factor in oil yield. It can be stated that the moisture content appears to be more effective in the oil extracted from BSF larvae. Based on the results, the models from Equations 4 to 6 are appropriate for predicting the oil yield within the chosen experimental range. Table 8 shows the actual and predicted model values of the oil yield and moisture content. It indicates that the predicted values of oil yield are near the actual experimental values. Figure 6 depicts

Table 7: The model parameter for the percentage yield of extracted BSF oil in each variable

Parameters		**Oil Yield (%)**	
		Coefficient	**P-value**
Moisture content	Intercept	96.98476298	1.06E-08
	X’	-0.339097065	0.000874491
Extraction time	Intercept	95.537	2.78E-06
	X’	0.001433333	0.89888768
Feeding rates	Intercept	96.456	8.82E-09
	X’	0.00968	0.001852112

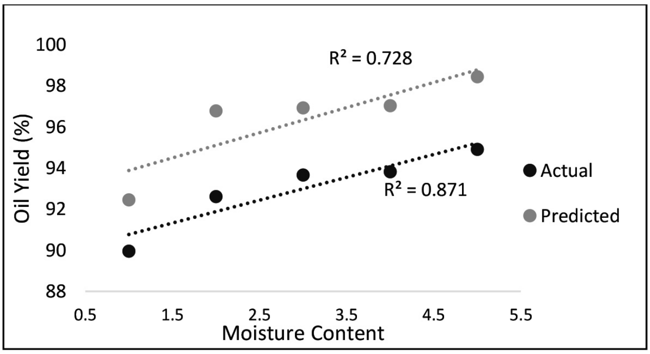

Figure 8: Plotted graph for actual and predicted versus moisture content for percentage yield of extracted BSF oil

Table 8: Experimental and predicted model values for different operating factors

Parameters	**Oil Yield (%)**		**Error (%)**
	Actual	**Predicted**	
Moisture content	89.95	92.45	2.78
	92.61	96.78	4.5
	93.66	96.92	3.48
	93.82	97.03	3.42
	94.9	98.44	3.73

a graph of actual and predicted oil yield versus moisture content.

Table 9 also shows the ANOVA for the percentage yield of BSFL oil in each variable. According to Table 9, the significant F value for moisture content and feeding rates when compared to the percentage of oil yield was less than 0.05. It means that the data and results are very dependable. Meanwhile, the F value for the percentage oil yield in terms of extraction time is 0.89. Because the F value is greater than 0.05, there is a significant probability that the regression output is not due to random chance or the null hypothesis.

Response Surface Methodology (RSM)

According to Table 9, the F values for moisture content, extraction time and feeding rates had the greatest impact on the percentage yield of BSFL oil. The effect of extraction time on the percentage oil yield was insignificant because the regression was unreliable due to insignificant R^2 and adjusted R^2 values. Figures 9 to 11 show the combined effects of two significant variables on the percentage of oil yield. As a result, the three-level factor Box-Behnken method was used to determine the optimal conditions for oil yield percentage.

A three-level, three-factor Box–Behnken design was used to predict the optimal conditions for oil yield using the FS and MPT as a function of several parameters, including moisture content, extraction time and feeding rates. The 3D model predicts the percentage of oil yield elimination plots for the three variables.

The Combined Effect of Feeding Rates and Moisture Content at Constant Time

According to Figure 9, the three-dimensional plot, feeding rates and moisture content significantly impact the percentage of oil yields. The moisture content of extracted oil

Table 9: The analysis of variance (ANOVA) for the percentage yield of extracted BSFL oil for each of the variables

Parameters		**df**	**Significance F**
			Oil Yield (%)
Moisture content	Regression	1	
	Residual	3	<0.01*
	Total	4	
Extraction time	Regression	1	
	Residual	3	0.89
	Total	4	
Feeding rates	Regression	1	
	Residual	3	<0.01*
	Total	4	

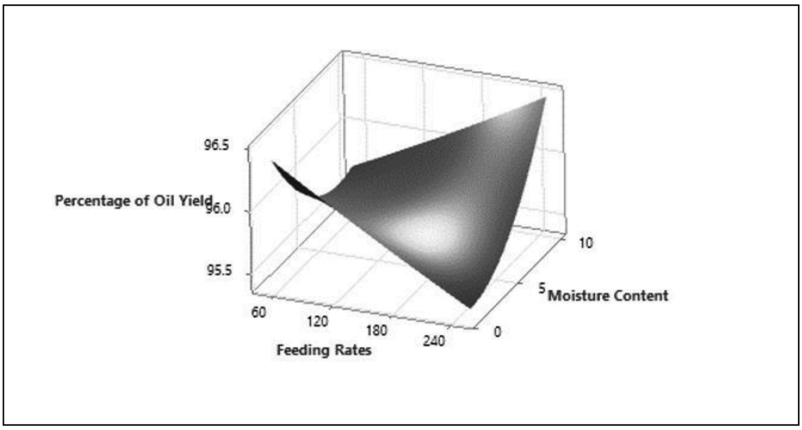

Figure 9: The 3D plot of the oil yield and moisture content of extracted oil at constant time

decreases proportionally as the feeding rate of larvae increases across the entire range of feeding doses used in this study. As a result, the maximum feeding rate of larvae is 150 mg/l/d, as shown in the 3D plot. This means that the higher the feeding rates, the more oil yield will be produced, provided that the moisture content is kept at less than 10%.

According to Nyakeri *et al.* (2019), when fed at 200 mg/l/d or higher, increasing feeding rates on oil yield resulted in higher larvae yield and shorter development time. Larvae growth and yield are minimal at lower feeding rates. Moreover, the feeding rates for larvae do not affect the moisture content. However, as the feeding rates increase, the oil yield percentage increases when the moisture content is kept below 10%. Hence, this proves that the feeding rates also significantly affect the percentage of oil yield when the moisture content decreases. In a nutshell, the combined effect was more significant at the two factors: Feeding rates and moisture content. When any of the factors increases, so does the percentage of oil yield.

The Combined Effect of Moisture Content and Extraction Time at Constant Feeding Rates

Figure 10 depicts the combined effect of moisture content and extraction time when the percentage of oil yield was observed from the three-dimensional plot. The extraction time was found to be longer at lower percentages of moisture content. However, as the moisture content increases, the extraction time decreases and the percentage of oil yield decreases. As a result, moisture content significantly impacted the percentage oil yield at any extraction time. According to Hasno *et al.* (2020), the higher the percentage of oil yield, the lower the moisture content. The percentage of oil yield, on the other hand, does not increase regardless of the increase in moisture content at any extraction time. It can be explained that a higher moisture content of 9.99% has achieved the maximum extraction time of 1 hour. In my outlook, the moisture content surface coverage has reached the maximum extraction time within 1 hour. As a result, it is possible to conclude that the percentage of oil yield is unaffected by the extraction time of 60 to 90 minutes.

The Combined Effect of Extraction Time and Feeding Rates at A Constant Moisture Content

Figure 11 depicts the percentage of oil yield at constant moisture content as a function of extraction time and feeding rates. It was discovered that as feeding rates were reduced, the percentage of oil yield also decreased. At feeding rates of 50 mg/l/d, the percentage of oil yield was reduced after 50 minutes of extraction.

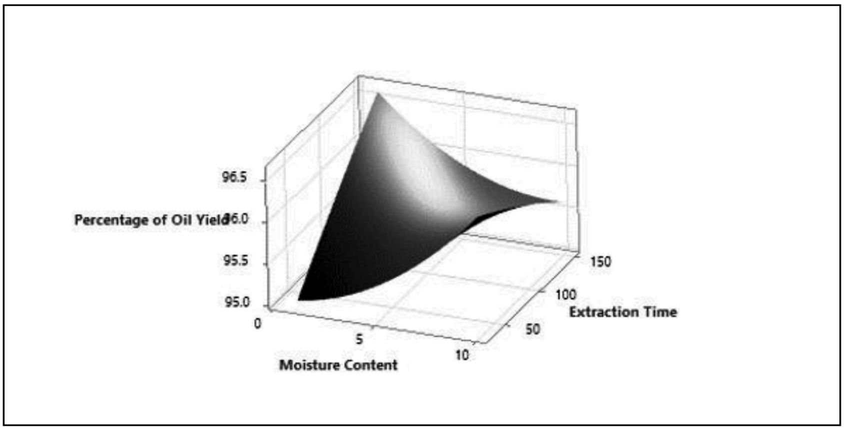

Figure 10: The 3D plot of the moisture content and the extraction time at constant feeding rates

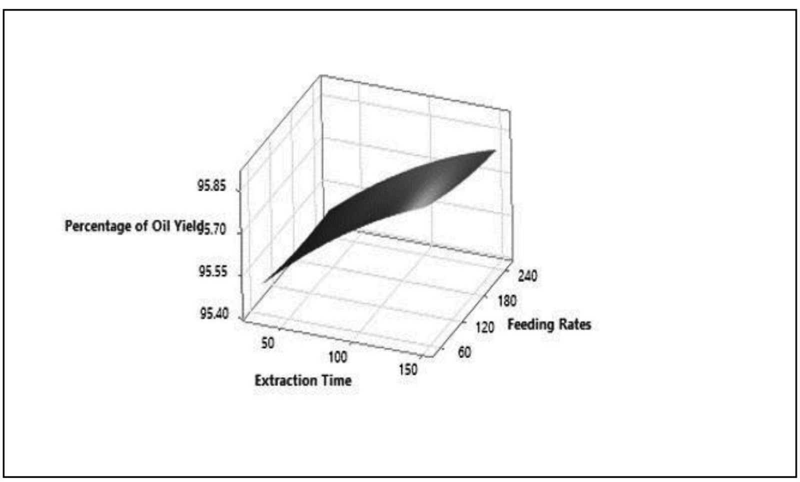

Figure 11: The 3D plot of the extraction time and the feeding rates at constant feeding rates

Furthermore, the figure shows that the percentage of oil yield does not increase as the extraction time increases. At lower feeding rates, the percentage of oil yield is nearly the same for extraction times of 90 minutes, 120 minutes and 150 minutes. In summary, the oil yield percentage was highly dependent on feeding rates but not on extraction time ranging from 30 to 150 minutes.

Conclusion

The study valued using different substrates by feeding the BSF with MPT and FS substrates to evaluate the quality of extracted oil composition using the mechanical-press method. MPT was more appealing to BSF larvae than FS. Fourier transform infrared spectroscopy (FTIR) analysis for both substrates reveals a similar high absorbance but High-Performance Liquid Chromatography (HPLC) analysis reveals glutamic acid to be the most abundant amino acid in both substrates. Furthermore, the regression analysis reveals that moisture content and feeding rate are the most important parameters with P and F values less than 0.05. Based on Response Surface Methodology (RSM), the best parameters for oil yield were moisture content and feeding rates. This provided more understanding into possible differences in BSF responses that can be ascribed to either the species' prior exposure to the MPT substrate or the FS substrate in producing high-quality oil. This study also revealed that BSF larvae could both survive and grow on a wide range of organic products, and yet not all organic materials are suitable for the growth and production of BSF production in captives as it affects both the quantity and quality of yield.

Acknowledgements

This research was funded by the GPK UiTM research grant (Code: 600-RMC/GPK 5/3 (246/2020).

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