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Analysis of Process Variables Effect on The Efficiency of Soxhletation Extraction of Larvae Oil (*Hermetica illucens)* using Response Surface Methodology

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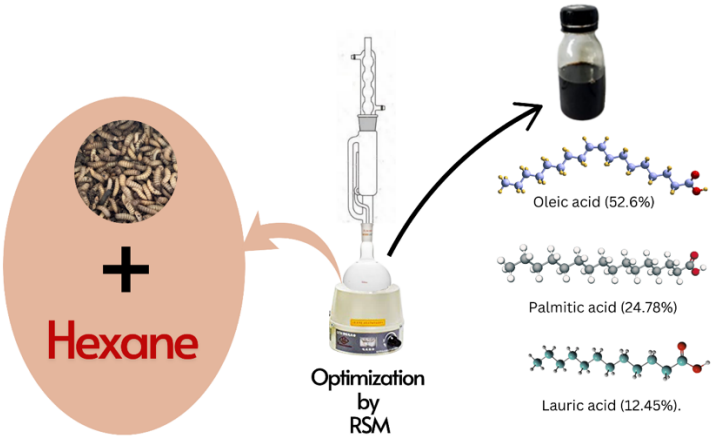
GRAPHICAL ABSTRACT



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ABSTRACT

Maggot or Black Soldier Fly (BSF) larvae can be used as a source of oil because they have a high lipid content. This study optimized the maggot oil extraction process using the soxhlet method with the assistance of the response surface methodology (RSM) using the Central Composite Design (CCD) research design. Optimization was done with variations in the ratio sample/solvent (1:7, 1:10 and 1:13 g/mL) and extraction time (120, 240 and 360 minutes). The results of the significance test using ANOVA showed that the sample solvent comparison had an insignificant effect (p-value: 0.060). In contrast, the extraction time has a significant impact (p-value: 0.000) on the maggot oil harvest yield. Different analyses showed a significant quadratic interaction between the sample/solvent ratio (p-value: 0.002) and a significant interaction between the sample/ solvent ratio and extraction time (p-value: 0.008). The recommended optimal conditions based on optimization using RSM are a combination of a sample/solvent ratio of 1:8.5 and an extraction time of 360 minutes with a predicted oil yield of 32.11%. Meanwhile, experimental validation at the recommended optimal conditions produced a yield of 38.68% or greater than the predicted value. The characteristics of the maggot oil obtained had a density of 0.9493 g/mL, a viscosity

of 0.92 Pa.s, water content of 0.0847% w/w, and free fatty acids of 4.56%. The results of GC-MS analysis of maggot oil showed a total of 9 compounds, with the 3 most abundant compounds being oleic acid (52.6%), palmitic acid (24.78%), and lauric acid (12.45%).

1. INTRODUCTION

BSF (Black Soldier Fly) larvae, mainly called maggots, originate from BSF fly eggs in the second phase of metamorphosis after the egg phase and previously the pupa phase, which then turns into adult flies [1]. Maggots utilize organic waste as nutrients in their growth. Maggot cultivation does not require high costs because growth nutrients are abundant in Indonesia. Based on the National Waste Management Information Data System, in 2024, the amount of waste in Indonesia was 18.35 million tons, and only 41.32% was managed. Based on data from the Ministry of Environment and Forestry in 2023, waste in Indonesia was dominated by organic waste (41.6% food waste), so maggot cultivation can help reduce the amount of waste in Indonesia. Meanwhile, in terms of the nutritional content of the maggot's body itself, in addition to containing protein, carbohydrates, ash, and fiber, maggots also have a high fat content, which is 21-25% [2] even up to 57.8% [3] depending on the oil extraction method, nutrition, and growth conditions. Given the high-fat content in maggots, in addition to reducing the volume of organic waste, BSF maggots can also be used as a source of oil.

Several studies have stated that maggot oil contains main compounds in the form of lauric acid (28.8−76.13%) and linoleic acid (~11.1%), as well as other compounds such as oleic acid, palmitic acid, myristic acid, and stearic acid [4]. However, the fatty acid profile in maggot oil can be modified based on differences in the bottom layer of maggot feed during growth [5]. The main compounds in maggot oil, both lauric acid and linoleic acid, can be utilized in various pharmaceutical products, food and beverages, cosmetics, body care, soap and detergents, plastics, and textiles [3, 6].

Maggot oil can be obtained through an extraction process using several methods and solvents. Commonly used solvents are hexane and petroleum ether. At the same time, the standard techniques used are Microwave Assisted Extraction (MAE) and soxhletation. Maggot oil extraction with petroleum ether solvent produces lower yields than hexane [7]. Meanwhile, maggot oil extraction using the microwave-assisted extraction (MAE) method with n-hexane solvent produces a yield of 30.22% [3], lower than using the soxhletation method with the same solvent with a yield of 40.34% [7]. The soxhletation method for maggot oil extraction is more effective because the solvent can be used repeatedly. Extraction of maggot oil using 70% n-hexane solvent using the MAE method produced yields of 30.27−30.38% (1:4 g/mL), 19.18−20.91% (1:6 g/mL), and 17.00−17.35% (1:8 g/mL) [8]. Meanwhile, extracting maggot oil using 1:10 n-hexane solvent using the soxhlet method produced a yield of 32.51±0.39% [9]. The extraction time and sample/solvent ratio determine the maggot oil yield produced. The longer the extraction time, the more oil is produced, and a proper ratio ensures sufficient solvent to dissolve all available oil in the sample [8]. A too-low ratio can lead to incomplete extraction, while a too-high ratio may be uneconomical and generate excessive solvent waste. Although Soxhlet extraction of maggot oil has been studied, the application of Response Surface Methodology (RSM) to optimize the combined influence of maggot flour to solvent ratio and extraction time on extraction efficiency is novel. This research utilizes RSM to systematically investigate these key variables and identify optimal conditions for maximizing oil yield.

2. EXPERIMENTAL METHODS

2.1 Material

Maggots originating from Jaya Larva East Lampung were fed food waste feed until they were 7−14 days old (water content 4−7%). They were then dried unblemished using a frying pan until dry and mashed into a fine powder, and technical n-hexane was used as the extraction solvent.

2.2 Method

2.2.1 Maggot oil soxhlet extraction

Maggot Flour Wrap using gauze paper with sample/solvent ratio of 1:7, 1:10 and 1:13 (g/mL) with n-hexane volumes of 420, 600, and 780 mL. Meanwhile, the time variations were 120, 240 and 360 minutes at 70 °C. The distillate oil obtained from the soxhlet extraction was concentrated using a rotary evaporator at 70–80 °C. This temperature range is chosen because it is above the boiling point of hexane (69°C), allowing the hexane to evaporate and separate from the maggot oil efficiently. The maggot oil obtained was centrifuged (3000 rpm) for 5 minutes at room temperature to increase its clarity.

Figure 1. Sample preparation and maggot oil extraction process using the soxhlet method.

2.2.2 Extraction Process Optimization

Optimization uses a statistical method called Response Surface Methodology (RSM) and, more specifically, Central Composite Design (CCD). This optimization describes a way to see the factors that affect the yield of maggot oil for extraction. The RSM method requires the creation of an empirical model to optimize process parameters and analyze factor interactions using experimental quantitative data. The CCD design includes factorial and axial paths centred around a central point to assess experimental error [11]. This study tested 2 variables: ratio (w/v) (maggot flour/hexane) and extraction time. Composite design was used in this study to test and improve maggot oil extraction.

2.4 Maggot oil's characteristics analysis

Extracted maggot oil is tested for its physical characteristics, including density and viscosity, and chemical characteristics, including free fat content and water content. A GC-MS test is also used to determine the compounds contained.

3. RESULTS AND DISCUSSIONS

3.1 Maggot oil extraction results

The yield of maggot oil produced based on variations in the ratio (w/v) and extraction time ranged from 19.21% to 32.11% (Table 1). The highest yield was produced from variations in the ratio (w/v) of 1:10 g/mL and an extraction time of 360 minutes with a yield of 32.11%. Meanwhile, the lowest yield was produced from variations in the sample/solvent ratio of 1:7 g/mL and an extraction time of 120 minutes with a yield of 19.21%. The longer the extraction time, the higher the yield because the longer the contact between hexane and the sample [12]. Meanwhile, increasing the volume of solvent will also increase the ability of hexane to dissolve components in maggot flour so that more maggot oil is extracted. However, even though a greater volume of solvent used initially can increase the extraction efficiency, excessive solvent volume will also make the sample solubility saturated and reduce the yield produced. This happens because the solvent has a limited capacity to dissolve a substance. If there is too much solvent, the substance to be extracted becomes too dilute and challenging to separate. In addition, the contact time between the solvent and the extracted material becomes shorter, so the dissolution process is imperfect. Other factors, such as extraction time, significantly affect the results [13, 14]. Therefore, it is essential to determine the optimal extraction conditions to obtain maximum results.

TABLE I. Experimental design for BSF maggot oil extraction using soxhletation with Central Composite Design (CCD).

Run Order	Ratio (w/v) (g/mL)	Time (minute)	Yield (%)
1	1:07	120	19.21
2	1:10	120	21.09
3	1:13	120	25.49
4	1:07	240	25.5
5	1:10	240	25.65
6	1:10	240	25.5
7	1:10	240	25.3
8	1:10	240	26.41
9	1:10	240	25.65
10	1:13	240	24.44
11	1:07	360	30.73
12	1:10	360	32.11
13	1:13	360	28.38

3.2 Statistical Analysis

TABLE II. Results of the Analysis of Variance (ANOVA) test.

Source	**DF**	**Adj SS**	**MS Adjectives**	**F-Value**	**P-Value**
Model	5	172,216	34,443	115.25	0.000
Linear	2	161,667	80,833	270.48	0.000
Ratio (sample:solvent)	1	1,500	1,500	5.02	0.060
Time	1	160,167	160,167	535.94	0.000
Rectangle	2	6,549	3,275	10.96	0.007
Ratio (sample:solvent)* (sample:solvent)	1	6,503	6,503	21.76	0.002
Time*Time	1	0.599	0.599	2.00	0.200
2 Way Interaction	1	4,000	4,000	13.38	0.008
Ratio (sample:solvent)*time	1	4,000	4,000	13.38	0.008
Error	7	2,092	0.299		
Lack of Conformity	3	0.892	0.297	0.99	0.482
Pure Mistake	4	1,200	0.3000		
Total	12	174,308			
R-sq = 98.88% R-sq (adj) = 97.94%					

Table 2 shows the results of the ANOVA analysis of maggot oil extraction. The coefficient of determination (R-sq) evaluates the developed model. If the R-sq value approaches 100%, the standard deviation (SD) becomes smaller, and the model better predicts the response [15]. It shows that the quadratic model has a relatively high R-sq value of 0.9888. This means that 98.88% of the experimental data is relevant, and the model cannot explain only 1.12% of the total variation.

Meanwhile, the sample/solvent ratio did not significantly affect the maggot oil harvest (p-value: 0.060). This means that it cannot be said that changes in the sample solvent ratio will always result in a significant difference in the maggot oil harvest (p-value <0.05) [15]. On the other hand, extraction time significantly affected the maggot oil harvest (p-value: 0.000), so the longer the extraction time, the more maggot oil can be obtained. Meanwhile, a different analysis showed a significant quadratic interaction between the sample/solvent ratio (p-value: 0.002). This indicates that the effect of changes in the sample solvent ratio on the maggot oil harvest is not linear. In other words, the impact of changes in this ratio is not constant but varies depending on the value of the ratio itself. On the other hand, there is a significant interaction between the sample solvent ratio and extraction time (p-value: 0.008). This shows that the effect of extraction time on maggot oil harvest yields varies depending on the sample/solvent ratio used.

3.3. RSM graph analysis

Figure 2 (a) is a contour plot showing the relationship between yield, sample/solvent ratio treatment and extraction time. The area in the darkest green shows a yield of >30%, while the area in the darkest blue shows a yield of >30%. Figure 2 (b) is a surface plot showing that changes in the sample/solvent ratio and extraction time affect the yield. In the graph, the peak area of the surface plot is depicted at a ratio (w/v) of 1:10 and an extraction time of 360 minutes with a yield of 32.11%. In general, surface plots help determine the optimal point in the sample variation:solvent ratio and extraction time to produce optimal yield. Figure 4 shows an optimal ratio (w/v) and extraction time to achieve maximum maggot oil yield.

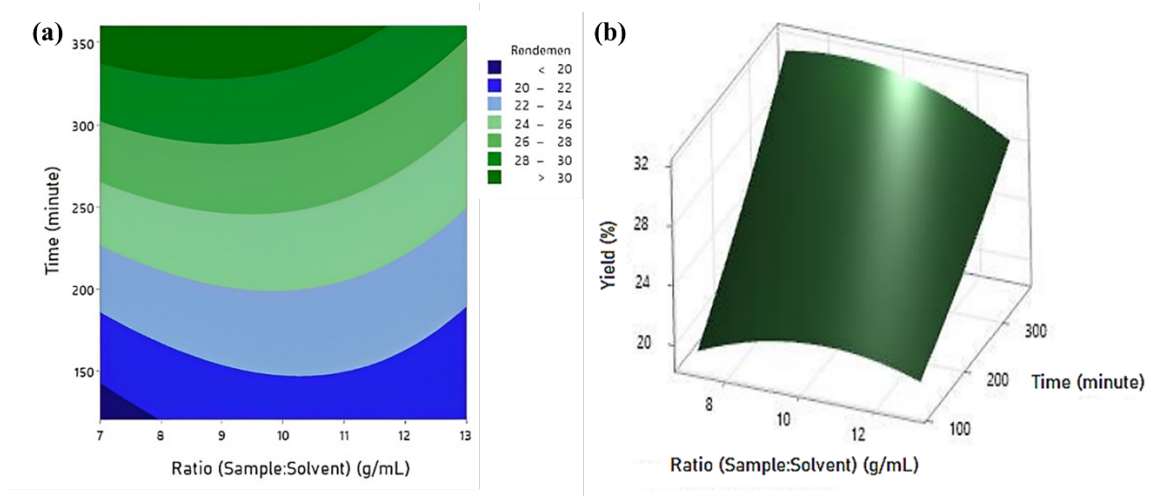

Figure 2. Contour plot (A) and surface plot (B) of extraction condition and yield.

However, the relationship between these variables is quite complex and non-linear. It is shown that the increase in one of the variations is not always directly proportional to the rise in visualization results. This is consistent with the previous ANOVA analysis (Table 2), which shows an interaction between the sample solvent comparison and extraction time.

3.4 Experimental validation of maggot oil extraction process optimization

The maggot oil extraction process was optimized using Central Composite Design (CCD), which purchased harvest results from 13 experimental combinations. Table 3 shows the optimization of maggot oil extraction using the Response Surface Methodology, with ratio (w/v) and extraction time as variables.

TABLE III. Prediction of optimal solution for maggot oil extraction process.

Solution	Ratio (w/v)	Time (minute)	Produce (%)	Composite Attraction
1	1:8.5	360	31.72	0.97868

The recommended conditions (Table 3) to obtain optimal maggot oil yields are variations in the ratio (w/v) of 1:1.8 and an extraction time of 360 minutes, with a predicted yield of 31.72%. The given treatment conditions are considered a perfect solution based on the optimization model used (composite desirability: 0.97868). After the optimization prediction of the maggot oil extraction process using Response Surface Methodology (RSM), experimental validation was carried out to verify the resulting yield. Table 4 shows the actual yield results of the extraction process based on the optimal conditions suggested by RSM.

TABLE IV. Experimental validation of optimal conditions of response surface methodology prediction.

Tasting	Ratio (w/v)	Time (minute)	Produce (%)
Maggot Oil	0.1:8.5	360	38.68

The experimental validation results of the optimal conditions suggested by RSM (Table 4) produced a harvest of 38.68%, higher than the predicted result of 31.72%. This means that the predicted value deviated by about 18.16% from the actual value based on the calculation of the relative difference. The magnitude of the difference is likely due to the influence of environmental factors during the extraction process or clouds in the model. However, the results show that the RSM model can provide a reasonably good estimate of the maggot oil harvest under the specified experimental conditions and can be the basis for process optimization in further research.

3.5 GC-MS analysis of maggot oil

The analysis of maggot oil using GC-MS (Figure 3) showed that were 9 compounds contained in maggot oil at a retention time of 10.57 to 25.32 minutes. The 9 fatty acid compounds contained in maggot oil (Table 5) consist of 7 saturated fatty acid (SFA) compounds in the form of lauric acid, myristic acid, palmitic acid, tetracosahexane acid, tetramethyl heptadecane acid, octadecanoic acid, and isostearic acid. It also consists of 2 unsaturated fatty acid (MUFA) compounds: oleic acid and palmitoleic acid. The 3 main compounds in the maggot oil produced are oleic acid ($CH_3(CH_2)_7CH=CH(CH_2)_7COOH$) (52.6%), palmitic acid ($CH_3(CH_2)_{14}COOH$) (24.78%), and lauric acid ($CH_3(CH_2)_{10}COOH$) (12.45%).

The composition of maggot oil is influenced by the type of waste used as the bottom layer of feed. The feed substrates from waste rapeseed cake and maggot oil shrimp contain the highest oleic acid (46.8 and 26.9%), compared to lauric acid (10 and 16.9%), palmitic acid (5.8 and 10.4%), and myristic acid (2 and 4.3%) [16]. Horse and fish (rainbow trout) feed substrates produce oleic and lauric acids that are not too different. The bottom layer of feed in the form of horse manure produces maggot oil composition of oleic acid (22.9%), lauric acid (28.1%), palmitic acid (22%), and myristic acid (6.7%) [17]. Fish feed substrate (rainbow trout) produces maggot oil composition of oleic acid (25.1%), lauric acid (28.6%), palmitic acid (12.6%), and myristic acid (6.1%) [18]. Meanwhile, the bottom layer of food, fed as fruits and vegetables, produces lauric acid as its primary fatty acid. The feed substrate in the form of fruits produces maggot oil with a composition of lauric acid (76.13%), myristic acid (8.46%), palmitic acid (6.98%), and oleic acid (4.97%) [19] and vegetable feed substrate produces maggot oil with a content of lauric acid (61%), myristic acid and palmitic acid are the same (9.5%), and oleic acid (5.7%) [20]. Other studies have added that maggot oil derived from maggots with a feed medium in the form of fruit waste contains lauric acid as the most abundant compound (34.28%), palmitic acid (19.25%), myristic acid (8.58%), and oleic acid (1.84%) [21].

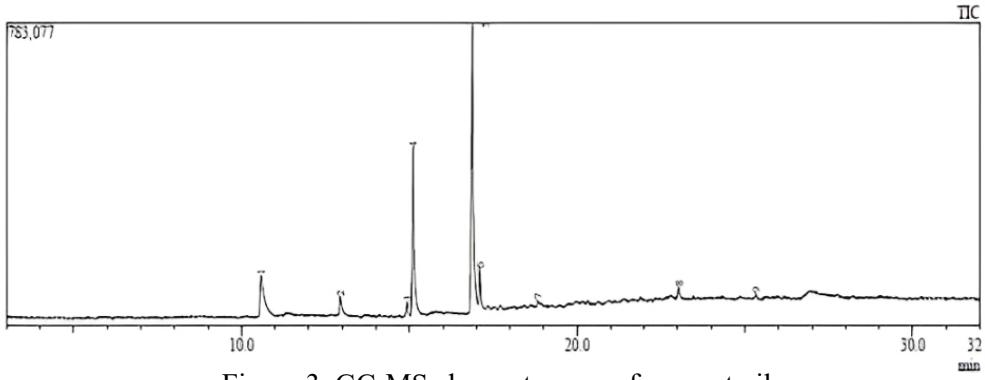

Figure 3. GC-MS chromatogram of maggot oil.

TABLE V. Maggot oil content by GC-MS analysis.

Peak	*Resistance Time (min)*	Area	*Mol Weight*	*Chemical Formula*	*Componen Name*	Result (%)
1	10,576	759986	214	$C_{13}H_{26}O_2$	Dodecanoic acid,Methyl laurate.	12,45
2	12,939	145545	242	$C_{15}H_{30}O_2$	Tetradecanoic acid, methyl ester (CAS)Methyl myristate	2,38
3	14,934	67144	282	$C_{17}H_{32}O_2$	9-Hexadecenoic acid, methyl ester, (Z) (CAS) Methyl palmitoleate	1,10
4	15,107	1512824	348	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester (CAS)Methyl palmitate	24,78
5	16,878	3210892	379	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester(CAS) Methyl oleate	52,60
6	17,099	279405	322	$C_{19}H_{38}O_2$	Heptadecanoic acid, 16-methyl-,methylester (CAS) Methyl isostearate	4,58
7	18,831	31189	277	$C_{19}H_{36}O_3$	Octadecanoic acid, 10-oxo-, methyl ester(CAS) Methyl 10 Oxooctadecanoate	0,51
8	23,028	80265	290	$C_{30}H_{50}$	2,6,10,15,19,23-Hexamethyl 2,6,10,14,18,22,- Tetracosahexaene	1,31
9	25,326	17257	242	$C_{21}H_{44}$	Heptadecane, 2,6,10,15-tetramethyl-(CAS) 2,6,10,15-Tetramethylheptadecane	0,29
		6104507				100

3.6 Characteristics of extracted maggot oil

Based on the results of the characteristic test (Table 6), maggot oil has a density of 0.9493 g/mL. The density of maggot oil varies due to several factors, such as the selection of extraction methods, maggot growth media, oil processing, temperature, and fatty acid profiles. Some research reported that maggot oil has a variable density, among others 0.913 g/mL [22], 0.913 (crude oil), 0.908 g/mL (after refining) [6], and 0.883 g/mL [23]. The oil sample's fatty acid profile also shows the oil's density. The saturation level of maggot oil containing high saturated fatty acids generally has a high density. In other words, the more saturated fatty acids in the oil, the higher the density of the oil [23].

Meanwhile, the viscosity of the extracted maggot oil is 0.92 Pa.s. Similar to the density value, the viscosity value of maggot oil also varies. Viscosity can increase with increasing carbon atoms and decrease with unsaturated bonds [24], so maggot oil has a higher unsaturated fatty acid content and viscosity. The extracted maggot oil has a water content of 0.0847% and a free fatty acids (FFAs)

content of 4.56%, which was determined based on the molecular weight of oleic acid as the most significant component in the extracted maggot oil.

TABLE VI. Maggot oil's characteristic.

Parameter	Results	
Density	0.94934	g/mL
Viscosity	0.92	Pa.s
Water content	0.0847	% b/b
Free fatty acids	4.56	%

Figure 4. Extracted maggot oil.

Although the water content of maggot oil has met the SNI 3741-2013 cooking oil standard (max. 0.15% w/w), maggot oil is not suitable for use as cooking oil or food ingredients because its free acid percentage exceeds the SNI 01-3741-2002 cooking oil standard (max. 0.3%) [25]. Free fatty acids are products of oil and fat hydrolysis [26]. Free fatty acids indicate that the oil has undergone triglyceride (neutral fat) breakdown into glycerol and free fatty acids [27]. This process is caused by the lipase enzyme and is influenced by temperature and humidity [28]. So, the high FFAs content may be due to the influence of temperature during extraction and the relatively long extraction time.

4. CONCLUSIONS

Through experiments with variations in the ratio of solvent/samples and extraction time, the optimal conditions were found to produce the highest oil yield. Analysis of the resulting oil showed that the main fatty acid content was oleic, palmitic, and lauric acids, which have broad application potential in various industries. The results of this study provide an important contribution to the development of BSF utilization as a sustainable source of animal oil and open up opportunities for further research related to BSF oil process optimization and characterization.

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