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Biodiesel Production from *Rhynchophorus ferrugineus* Larvae Oil: Physichochemical Properties and Acid Composition of Oil as Affected by Oil Extraction Protocol

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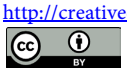

Abstract

Biodiesel, a renewable energy source made from natural oils and fats, can be produced using white raffia larvae as a raw material. These larvae have a high lipid content and a short life cycle, making them suitable for this purpose. One crucial step in biodiesel production is oil extraction, and this study aimed to investigate how the extraction protocol affects the fuel properties of the oil. To study the impact of solvent type, solvent volume, and residence time on oil yield, 200 grams of *Rhynchophorus ferrugineus* were used in a Soxhlet extractor. The researchers examined the physicochemical properties and fatty acid composition of the crude grease using the European biodiesel standard (EN14214) and gas chromatography methods, respectively. The study found that hexane as a solvent produced the highest oil yield (64.44%) during a four-hour extraction period with a solvent ratio of 300 ml. Furthermore, the hexane-extracted oil had the highest iodine number (3.02 g/100 g) and cetane number (55.69). These values indicate favorable properties for biodiesel production. The *Rhynchophorus ferrugineus* larvae oil proved to be a rich source of monounsaturated fatty acids (76%), which were found to be significantly affected by the solvent type. Based on quality assessment, *Rhynchophorus ferrugineus* can be suitable for biodiesel production. In summary, under the given operational conditions, hexane is the most suitable solvent for *Rhynchophorus ferrugineus* oil extraction for biodiesel production. Further research in optimizing the extraction process can contribute to the efficient utilization of renewable energy sources like white raffia larvae for biodiesel production.

Keywords

Biodiesel, Extraction Process, Fatty Acid Composition, Physico-Chemical Properties, *Rhynchophorus ferrugineus* Larvae

1. Introduction

The energy model that currently prevails is highly dependent on the usage of fossil fuels and supports various sectors such as transportation, industry, and agriculture, among others [1]. However, this model has become less and less viable due to the reduction in the non-renewable energy source, its increasing price, and the fact that this type of fuel favors an elevation in greenhouse gas emissions, some of which have been shown to have an impact on people's health as they are related to various types of cancer [2] [3] [4]. As stated by the United Nations' Sustainable Development Goals (SDG), regarding sustainable energy access (SDG7) and climate change (SDG13), several countries have started to take actions to reduce carbon emissions by at least 43% by 2030 and to not reach an increase in global temperature of 1.5˚C between 2030 and 2050 (IPCC, 2018) [5]. The supply of oil has led to geopolitical tensions, and concerns about its scarcity and environmental impact have sparked significant interest in alternative energy sources like solar, wind, and biomass energy [1]. Biomass biofuels are considered one of the most intriguing alternative fuels to fossil fuels [6]. However, they are still a topic of controversy on a global scale. The initial generation of biofuels utilizes edible plant matter, which creates a competition issue between the food and biofuel industries [7]. To overcome this problem, current research in bioenergy, specifically biofuel, is focusing on finding new raw materials with superior physicochemical properties. Non-edible plant matter such as Jatrophaor castor oil [8], as well as microalgae [9] [10], are being developed for biodiesel production. Nevertheless, the extraction of oil from these seeds requires significant effort due to their low efficiency, and they also have a lengthy renewal process.

As a substitute fuel, biomass biofuels appear to be one of the most interesting alternatives to fossil fuels [11]. Currently, there is a new source of raw materials for insect-based products, such as the *Rhynchophorus ferrugineus*, also known as PW [12]. This insect can be found in various rural areas in Cameroon, as well as in other parts of Africa and Asia [12]. Although PW is known to cause damage to palm, coconut, sago, and date plantations, the industry has been using insecticides to control their population [13]. However, it is interesting to note that *Rhynchophorus* has potential as a biodiesel feedstock [14] due to its high lipid content compared to other insects, ranging from 31.4% to 69.78% of its dry weight. Additionally, these larvae have a high reproductive capacity and require relatively little space for breeding, with each crossbreed laying 250 - 500 eggs [12].

One of the important steps in biodiesel production is oil extraction. The three most commonly employed conventional oil extraction methods are mechanical, chemical (solvent), and enzymatic [15]. In commercial oil extraction, solvent extraction and mechanical pressing are the most commonly used methods. Most of the time, oilseed extractions are made using mechanical processes, which are time- and energy-consuming. However, solvent extraction is more efficient in terms of oil recovery [16]. In solvent extraction, the soxhlet method is the most known because it is simple to use and economical [17]. The choice of solvents for extraction plays an important role in the final outcome. Solvents used for oil extraction can affect the oil yield [18], fatty acid profiles [19], and physicochemical properties [20]. In fact, for some known raw materials like jatropha [21], turber [22], etc., the oil extraction protocols are already known.

Rhynchophorus ferrugineus, unlike other species, does not have sufficient research on its oil extraction process. Although there have been some studies on extracting oil from *Rhynchophrus errugineus* using physical extraction methods [14], further investigation is needed to establish a suitable oil extraction process specifically for the larvae of this species. Hence, the objective of this study is to evaluate how different oil extraction protocols impact the fatty acid composition and physicochemical properties of *Rhynchophrus errugineus*.

2. Materials and Methods

WPL used in this experiment were collected from the palm plantation in the forests of East Cameroon. After collection, the larvae were dried in an oven at 70˚C for 1 hour. Next, the larvae were dried and subsequently processed in a grinding mill to obtain a fine powder. This powder was further utilized for extracting oil by conducting an analysis of the impact of different solvents, time, and solvent volume on the physicochemical properties and fatty acid of oil.

2.1. Oil Extraction

2.1.1. Solvent Type

In order to study the effect of solvent type, two different solvents (n-hexane and petroleum ether) were independently used. Grounded larvae (5 g) were placed in a 250 cm^3 laboratory Soxhlet apparatus made in a porous cellulose thimble (**Figure 1**). Oil was extracted by repeated percolation using the above two organic solvents as described by Adejumo *et al.* [23].

2.1.2. Solvent Ratio and Resident Time

Twelve extraction experiments were performed to evaluate the impact of extraction time and solvent volume on extraction yield. The parameters of extraction time and solvent volume were varied based on the experimental design presented in **Table 1**.

After the extraction, the flask containing the solvent and oil mixture was removed, and the solvent was evaporated. The extracted oil was then dried at 100˚C for 60 minutes in an oven to eliminate any remaining solvent residue. The mass

Figure 1. Solvent extraction protocol.

Table 1. Experimental design summary.

Solvent type	Residence time (h)	Solvent quantity(ml)
Petroleum ether	4	300
		250
		200
	6	300
		250
		200
Hexane	4	300
		250
		200
	6	300
		250
		200

of the dried oil was measured, and the oil extraction yield was calculated using Equation (1).

Formula (1).

$$O_y = \frac{W_b - W_a}{W_b} \quad (1)$$

where:

O_y = oil yield %;

W_b = weight before extraction;

W_a = Weight after extraction.

2.2. Fatty Acid Composition and Chemical Characteristic of White Palm Weevil (WPL) Crude Grease

2.2.1. Fatty Acid Composition Analysis of Crude Grease

Fatty acid profiles of WPL oil were performed in the Biochemistry Laboratory, Friedrich Alexander University of Erlangen-Nuremberg, Germany using gaz chromatography method according to the procedure of AOAC, (2000) as described by Jayanegara *et al.* [24].

2.2.2. Chemical Characteristic

a) Acid Index

The acid index was measured by titration with potassium hydroxide (ASTM D-664) as described by Asmare and Gabbiye [25]. For acid index determination, the oil samples were titrated in triplicate using 0.5 g ± 0.1 g in a 125 ml Erlenmeyer. Then 10.0 mL of ethanol and ethyl ether (1:1 mixture (v/v) and phenolphthalein drops (0.1% in ethanol) as the indicator were added. The titration was performed with an aqueous solution of KOH (0.01 mol·L^{-1}), properly standardized with a solution of potassium biphthalate. The volume V_0 of KOH used was noted at the end of the titration and the acid index was calculated using equation 2:

$$I_a = \frac{V_1 - V_0}{m} \times 56.1 \times T \tag{2}$$

b) Iodine Number

The Iodine Number (IN) was measured according to ASTM D664, NFT60-204 standard. For iodine number determination, approximately 0.5 g of WP larval oil was placed in a 250 ml conical flask. Cyclohexane: glacial acetic acid (1:1 V/V) (20 ml) and 10 ml of Wijs reagent solution were added into the flask. The entire solution was thoroughly mixed and kept in the dark for an hour. About 15 and 100 ml of 15% Potassium iodide (KI) and distilled water were added to the flask and the solution was titrated against 0.1 M sodium thiosulphate ($Na_2S_2O_3$) solution to a colorless end point using starch as an indicator. The IV was calculated using Equation (3.3).

$$\text{Iodine Value g}\cdot\text{I}_2\cdot\text{g}^{-100}\text{ oil} = \frac{B * S * M * 126.9 * 100}{W_g * 1000} \tag{3}$$

where B = volume 0.1 M sodium thiosulphate used in titrating the blank, S = volume of 0.1 M sodium thiosulphate used in titrating the sample, 126.9 = molar mass of iodine, M = Molarity of sodium thiosulphate, W = sample weight in gram.

c) Saponification Value

The saponification number was determined according to Ogbunuga *et al.*, [26]. White palm larvae (WPL) oil weighing 5 g was placed in a flask and 50 ml of 1.0 M ethanolic potassium hydroxide (KOH) was added. The flask was connected to a reflux condenser and was refluxed for 1 h until the solution became clear. A blank sample containing only 50 ml ethanolic potassium hydroxide was similarly treated as the sample. The solution was then titrated to a faint pink color endpoint against 1.0 M hydrochloric acid (HCl) using a phenolphthalein indicator. The saponification value (SV) was calculated using Equation (3.5):

$$\text{SV (mg}\cdot\text{KOH}\cdot\text{g}^{-1}\text{) oil} = \frac{(A - B) * N * 56.02}{W} \tag{4}$$

where A = Blank ethanolic HCl volume in ml, B = sample ethanolic HCl volume in ml, N = Normality of HCl, W = Weight of sample/oil in grams.

2.2.3. Physical Characteristic

Physical characteristics of white palm larvae such as cetane number (*CN*) and

calorific value (*CV*) were determined. The cetane number was determined by applying Equation (5), as proposed by Molla and Nigus [27], which relies on an empirical formula incorporating the saponification value and the iodine number.

$$CN = 46.3 + (5458 * SV) - (0.225 * IN) \quad (5)$$

where *CN*: Cetane number;

SV: Saponification number;

IN: iodine number.

Calorific Value (*CV*), which represents the quantity of heat released by the complete combustion of biofuel, was calculated from Equation (6) [28].

$$CV = 49.43 - (0.04 * SV) - (0.015 * IN) \quad (6)$$

where *CV*: calorific value (MJ/Kg);

SV: Saponification number;

IN: iodine number.

2.3. Statistical Analysis

Each experiment was repeated three times. Data for oil extraction were subjected to the analysis of variance (ANOVA test) Duncan's post hoc test ($p \leq 0.05$) was used to determine the homogeneity subsets whenever significant differences existed among the treatments. The statistical software used was SPSS for Windows, version 2.0.

3. Results and Discussion

3.1. Effect of Extraction Protocol on WPL Oil Extraction Yield

The design layout for WPL oil extraction and their responses on oil extraction yield percentage are presented in **Table 2**. It can be seen that the values of oil yield vary from 56.34 ± 2.62 to 64.44 ± 4.39. The oil yields recorded during this work were higher than the oil yield obtained by chemical extraction from black soldier fly larvae (30.1%; 29.1%; and 32.2%), which are the most requested insects for biodiesel production [29]. Additionally, they also outperformed the yield (38% to 46%) reported by Atabani *et al.* [15] for rapeseed oil extracted using Soxhlet extraction. On the other hand, the oil yield was significantly ($p < 0.05$) affected by time, solid-to-solvent ratio, and type of solvent.

1) Effect of Solvent Type and Time on Extracted Yield

The oil yield was significantly ($p < 0.05$) affected by three factors: time, solvent type, and solvent volume. Based on this study, the optimal oil extraction times were 4 hours for hexane and 6 hours for petroleum ether. Hexane solvent produced the highest extraction yield (64.44 ± 4.39) compared to petroleum ether (56.89 ± 1.47) This can be explained by the low dynamic viscosity of hexane, which facilitates the extraction of oil from the cells [30]. The extraction time specifically had a significant impact on the yield of n-hexane and petroleum ether extracted WPL oil. According to **Table 2**, the oil yield percentage obtained through

Table 2. Solvent extracted Oil yield percentage.

Reagents	Time	Solvent volume (ml)	Oil extraction yield (%)
Hexane	4 h	300	66.44 ± 0.44[a]
		250	57.39 ± 2.16[ab]
		200	48.06 ± 2.1[bc]
	6 h	300	57.04 ± 1.72[c]
		250	56.88 ± 0.59[c]
		200	42.28 ± 0.23[bc]
Petroleum ether	4 h	300	56.89 ± 1.47[c]
		250	56.34 ± 2.62[c]
		200	50.56+1.5[ac]
	6 h	300	59.77 ± 1.03[cb]
		250	61.35 ± 1.11[b]
		200	35.48 ± 2.1 [cd]

a, b, c: means with the same superscript are not significantly diffrent at 5%.

petroleum ether extraction increased from 56.34 to 61.35 percent as the extraction time extended from 4 to 6 hours. This observation aligns with the study conducted by [31], which also found that the oil yield percentage rises with longer extraction times. However, the oil yield obtained from hexane extraction decreased with increasing reaction time, indicating that the maximum extraction yield with this solvent is obtained after 4 hours. This finding is in accordance with the research of Bayisa and Bullo, [32] on Croton macrostachyus seed oil extraction.

2) Effect of Solvent Volume on Extraction Yield

The results depicted in **Figure 2** show the impact of solvent volume on the extracted oil yield. The graphs indicate that increasing the amount of solvent from 200 to 250 ml led to a higher oil extraction yield for Hexane and petroleum ether solvents. This result can be explained by the increased concentration gradient between the solid and liquid phases leading to enhanced mass transfer. Similar findings were reported by Ntalikwa, [33] in their study on jatropha. However, increasing the solvent volume from 250 ml to 300 ml did not significantly improve the oil yield for hexane extraction at 6 hours and petroleum ether at 4 and 6 hours. The study found that the optimal oil extraction yield of 66.44% ± 0.44% was achieved with a solvent volume of 300 ml and a reaction time of 4 hours for hexane.

3.2. Effect of Solvent Type on Fatty Acid Composition of WPL Oil

The effect of solvent type (hexane and petroleum ether) used for extraction on the fatty acid composition of WPL oil is presented in **Table 3**.

The study found that oleic acid was the predominant fatty acid in both hexane-extracted oil (56.52%) and petroleum-extracted oil (58.04%). Palmitic acid

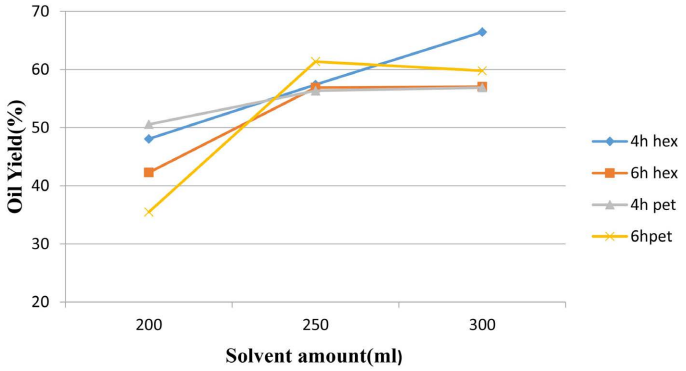

Figure 2. Effet of solid-to-sonvent ratio on the oil extraction yield of WPL.

Table 3. Fatty acid composition of palm weevil larvae oil according to the extraction solvent type.

Fatty acid group	Fatty acid type	Hexane	Petroleum ether	p
Saturated fatty acid	Palmitic (16:00)	13.50 ± 0.46^{b}	14.13 ± 0.24^{a}	0.006
	Lauric acid (12:00	0.59 ± 0.09^{a}	0.65 ± 0.16^{a}	0.120
	Heptadécanoic acid (17:00)	1.12 ± 0.19^{a}	1.32 ± 0.45^{a}	0.587
	Stearic acid (18:00)	4.07 ± 0.55^{b}	3.93 ± 0.28^{a}	0.048
	Myristic acid (14:00)	9.85 ± 0.24^{b}	9.27 ± 0.23^{a}	0.015
Polyunsaturated fatty acid	Linoleic acid (18:02)	8.48 ± 0.18^{b}	10.02 ± 0.35^{a}	0.004
	Linolenic acid (18:03)	0.36 ± 0.18^{a}	0.67 ± 0.10^{a}	0.109
Mono unsaturated acid	Oleic acid (18:01)	58.04 ± 0.87^{b}	56.52 ± 0.74^{a}	0.036
	Palmitoleic acid (16:01)	2.70 ± 0.45^{a}	2.50 ± 0.55^{a}	0.250

a, b: means with the same superscript are not significantly diffrent at 5%.

was the second-highest fatty acid in the oil and was significantly higher ($p < 0.05$) in petroleum-extracted oil (14.13%) compared to hexane-extracted oil. Myristic acid was also influenced by the solvent type and was higher in percentage in hexane-extracted oil (9.85%) compared to petroleum ether-extracted oil (8.45%). There were no significant differences in the levels of lauric, heptadecanoic, linolenic, or palmitoleic acid between hexane and petroleum-extracted oil. The presence of monounsaturated fatty acids (oleic and palmitoleic acid) was predominant in the oil from WPL. This finding aligns with the finding of Jedidi *et al.* [34] on Tecoma stans seed oil. The high concentration of monounsaturated fatty acids in the oil suggests that the biodiesel produced from it will have a high cetane number [35], leading to improved ignition and fuel emissions [36]. Additionally, unsaturation in the fatty acid chain enhances the cold flow property and offers better stability and a longer shelf life to the biodiesel [37]. Similar results have also been reported for para-rubber seed oil [38] and pumpkin oil [30] extracted using different solvents.

3.3. Effect on Physico-Chemical Properties of the WPL Oil

3.3.1. Acid Value

The solvent type had a significant impact on the acid value, as shown in **Table 4** ($p < 0.05$). The highest acid value was observed in petroleum extracted oil, with a measurement of 4.90 mg KOH/g. The acid value of the HEO (Hexane Extracted Oil) used in this study was similar to the value of 4.37 mg KOH/g reported for R. phoenicis larvae oil [39], but higher than the values of 0.4 mg KOH/g for Moringa stenopetala seed crude oil [31] and 0.53 mg KOH/g for jatropha oil [21]. However, the acid value in this study is lower than the value of 51.81 mg KOH/g reported by Atabani and colleagues [15] for WPL oil extract obtained with a mechanical press. This difference could be attributed to the fact that oil extracted with organic solvents tends to have a lower acid value compared to oil obtained through mechanical extraction [40].

3.3.2. Iodine Value

The influence of solvent type on the iodine value is evident from the data presented in **Table 4**. The iodine value was significantly affected by the choice of solvent ($p < 0.05$). Specifically, the use of hexane solvent resulted in the highest iodine value of 3.02 g/100 g, whereas petroleum ether yielded a lower value of 2.05 g/100 g. The iodine value is a measure of unsaturation and serves as an indicator of the amount of double bonds present in the oil, which is related to its susceptibility to oxidation. This finding is consistent with previous research conducted by Lesten and Kingsley [30], who also reported a difference in iodine values between hexane-extracted oil and petroleum-extracted oil from pumpkin seeds. Furthermore, the iodine values obtained in this study were lower than those reported by Tangka *et al.* [14], who extracted *Rhynchophorus phoenicis* oil using a mechanical press and obtained a value of 36.83 g/100 g. This difference can be attributed to the lower level of double bonding (polyunsaturated acid value of 8.85%) found in the fatty acid composition of the WPL oil in our study.

3.3.3. Cetane Number and Calorific Value

When R. phoenicis was extracted with hexane solvent instead of petroleum ether, the cetane number and colorific value increased (**Table 4**). The cetane number of WPL oil was higher than that of conventional diesel (50) and rapeseed vegetable (39) oil, [41] but lower than that of WPL mechanically extracted oil. This difference can be attributed to the high concentration of unsaturated fatty acids in the WPL oils obtained in this study. Oils with a higher content of saturated fatty acids have a higher cetane number and are more likely to self-ignite under compression [42].

4. Conclusion

The oil content of WPL ranged from 56.36% to 64.44% at the conclusion of this study. The results also confirm that n-hexane is a suitable solvent for the extraction of WPL oil, and the optimal conditions are as follows: extraction time of

Table 4. Effect of solvent type (hexane and petroleum ether) used for extraction on physico-chemical properties of WPL oil.

Extraction	Solvent	Parameters
	Hexane	Pretroleum ether
Acid value (mg/g)	3.58 ± 0.11[b]	4.90 ± 0.18[a]
Saponification value (mg/g)	535.00 ± 52.33[b]	699.50 ± 6.36[a]
Iodine number (g/100 g)	3.02 ± 0.21[a]	2.05 ± 0.21[b]
Cetane number	55.69	53
Calorific value (Mj/Kg)	27.44	20.68

a, b: means with the same superscript are not significantly diffrent at 5%.

4 h, a volume solvent of 300 ml, extraction temperature of 68 °C, and an oil yield of 64.44%. In addition, the study shows that WPL oil is a rich source of monounsaturated acids (oleic and palmitoleic acids) and palmitic acid, which is significantly affected by the solvent. Furthermore, the physicochemical properties of WPL oil in this study indicate low iodine and a high saponification value, which implies a high monounsaturated to saturated fatty acid ratio and might be an acceptable substitute for highly saturated oils such as olive oil and palm oil. The current finding demonstrated that the WPL oil has great potential to be utilized as a raw material for biodiesel production, and its full potential should be exploited.

Author Contributions

This work was carried out in collaboration among all authors. Author AFEA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JKT and Nsah-ko T managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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Conflicts of Interest

Authors have declared that no competing interests exist

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