



## Evaluation of *Raffia* Palm Weevil Larvae (*Rhynchophorus phoenicis*) as a Potential Biodiesel Resource

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### Authors' contributions

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

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### ABSTRACT

Ethical issues arise when arable agricultural land and heavy agronomic inputs are used to cultivate crops for energy production such as biodiesel. Alternative feedstocks for biodiesel production might solve the food energy competition scenario. White raffia larvae (*Rhynchophorus phoenicis*) appears as a credible option because of its high lipid content, a relatively short life cycle and its abundance in the tropical environment. This study investigated the use of *R. phoenicis* larvae for biodiesel production. *R. phoenicis* larvae was grown on raphia stipe for 21 days and used for crude grease extraction. The extracted crude grease was converted into biodiesel by acid-catalyzed esterification and alkaline-catalyzed trans-esterification. The physicochemical properties of crude grease and biodiesel were investigated using the European biodiesel standard (EN14214), and the American Society of Testing materials specifications. The defatted extraction residue was analyzed using the AOAC protocol. Results revealed that the cetane number, heating value, acid value and density of crude grease were found to be higher than those of rapeseed while kinematic viscosity and iodine values were smaller. The conversion yield of free fatty acids of crude grease into biodiesel reached

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85%. The fuel properties of biodiesel obtained are quite comparable with those of EN 14214 and ASTM standard. The defatted extraction residue exhibited high protein content (66,76%) and mineral which make it suitable for animal and human feed supplement. Therefore, it was concluded that, the *R. phoenicis larvae* can be used as valuable feedstock for biodiesel production and animal feed resources.

**Keywords:** *R. phoenicis larvae*; transesterification; physico-energetic properties; biodiesel.

## 1. INTRODUCTION

The development of industry, the expansion of urbanization and the increase of the world's populations have led to very high demands on fossil energy which cover 80% of global energy consumption [1]. Fossil fuels are present in all sectors of development and their availability signifies power for so many nations. However, fossil fuels are the main cause of all geopolitical conflicts in the world. Other inconveniences of fossil fuels include the need for long-distance transportation to the consumption points, concerns of climate change and the increasing international legislation governing their exploitation and use [2]. Moreover, these conventional energies, main sources of supply, are becoming increasingly scarce and expensive. The availability and prices of fossil fuels are not governed by the economic laws of demand and supply but by alignment to political ideologies and the urge to superpower positioning. The poor road network in most developing countries makes the distribution of fossil fuels impossible in some regions. In addition to its high cost, the production and use of these fossil fuels promote the emission and accumulation of greenhouse gases. These gases are the main causes of environmental pollution and climate change. The geopolitical tensions linked to the supply of oil, likewise the increasing scarcity and environmental concerns have generated much interest in alternative sources of energy such as solar, wind, and biomass energy [3]. As a substitute fuel, biomass biofuels appear to be one of the most interesting alternatives to fossil fuels [4]. However, it remains a source of many controversies at the international level. The first generation biofuels use edible vegetable matter as raw material. This use of edible vegetable matter poses a competition problem between the food industry and the biofuel industry [5]. To overcome this challenge, non-edible vegetable matter such as *Jatropha* or castor oil [6], microalgae [7,8], are being developed for biodiesel production. These inedible plant materials face their own challenge such as the used of arable land that could be devoted to

edible vegetable matter, the long live cycle and the used of limited water resources [8]. Attempts to cultivate these crops together with edibles crops in agroforestry programs have been developed in some countries like Brazil, Mali, Burkina Faso, Nigeria and Cameroon. Many of these attempts have remained at the pilot face level with promising results and little scaling up. A more economically feasible and environmentally friendly approach is the use of insects for biodiesel production [9]. In fact, interest in insects as lipid feedstock producers has increased, particularly due to their ability to accumulate large amounts of saturated fatty acids. The alternative of using insects also appears attractive because the defatted larval biomass can be used as a protein source for poultry, aquaculture, and livestock [9]. Insects such as the black soldier fly (BSF) (*Hermetia illucens* L.) which contain 23–47% of lipid [10,11,9] and 20–40% of lipid [12] are used as feedstock for biodiesel production. *Rhynchophorus phoenicis*, possesses numerous potentials as a biodiesel feedstock such as; high lipid content of about 31.4 to 69.78% of the dry weight when compared to other insects, and a short growth cycle duration estimated at 2 months from eggs to the adult larval stage [13]. *Rhynchophorus spp* is an insect that grows in almost all rural areas in Cameroon, as well as in other African and Asian countries. Interestingly according to Monzenga et al. [14] 43.8% of this larvae biomass is composed of fat which could be valuable non-food feedstock for biofuel production. In addition, these larvae have a high reproductive capacity (250 to 500 eggs laid per crossbreed) and require a small space for breeding [14]. Such a feed stock would considerably improve on energy access because its multiplication is very simple and the yield is quite high. Production of these larvae is not a net CO<sub>2</sub> pollution activity. However, its biodiesel potential has not yet been explored. Therefore, the aim of this research was to investigate the possible use of *Rhynchophorus phoenicis* larvae commonly known as white larvae of raffia beetle (WRL) as a biodiesel feedstock.

## 2. MATERIALS AND METHODS

### 2.1 Breeding of *Rhynchophorus phoenicis* Larvae

The WRL used in this experiment were raised following the methodology described by Monzenga et al. [14]. Five adult insects couples were trapped in the raffia palm bush located in Dschang University Research Farm and introduced into an aerated plastic jar containing a source of feed which was pieces of sugar cane chopped at 5 to 7cm. The ratio used was 1/1 (a female for a male). Ten days after the first mating, 260 young larvae were extracted and then introduced into a raffia palm stipe renewed after a week. After the 21 days of feeding, the WRL were separated from the residues, washed with distilled water, and heated on a hot plate at 100°C during 30 minutes.

### 2.2 Crude Grease Extraction

Crude grease was extracted from 1,3 kg of larvae mechanically using a vertical hydraulic press (Model CKT 2000AUF) and a perforated cylinder. After packing the larvae in a perforated cylinder of 20 cm diameter and 30 cm height, pressure was applied to the larvae through a piston until all the mechanically extractable liquid was separated from the cake at about 5 bar. This method was chosen instead of chemical extraction because of the intention to analyze the pressed caked as a possible supplement for human and animal nutrition. The use of chemical extraction could have introduced some chemicals into the cake and rendered it not good for human and animal nutrition. The liquid obtained from this extraction was centrifuged at 400 rpm during 10 minutes to separate crude grease from the larval liquid and impurities. The extraction yield (Ey) was determined using equation 1. The grease extracted was subjected to physicochemical analysis and used for biodiesel production.

$$Ey(\%) = \frac{\text{mass of crude grease extracted (g)}}{\text{mass of fresh larvae}} \times 100 \quad (1)$$

### 2.3 Fuel Properties of Crude Grease

The fuel properties of *R. phoenicis* crude grease produced were determined following the EN 14214 and ASTM specifications. The density of crude grease was determined by the hydrometer method (ASTM D-1298). The viscosity at 40°C

was determined with empirical viscometers, in which the limit velocity of fall ( $v$ ) of a marble with radius ( $r$ ) in a fluid with density ( $\rho$ ) were measured. The dynamic viscosity was determined through the law of Stokes by equation 2 as proposed by Okraonye and Ikewuchi [15].

$$\eta = \frac{g \times r^2 (\rho_{\text{marble}} - \rho_{\text{fluid}}) \times t}{18 \times d} \quad (2)$$

With:

$\eta$ : Dynamic viscosity in Pa. S (Pascal second);

$\rho_{\text{marble}}$ : density of the marble;

$\rho_{\text{fluid}}$ : density of the fluid (kg / m<sup>3</sup>);

$d$ : Distance traveled by a marble in meters (m);  $t$ : Time taken by a marble in second (s);

$r$ : radius of a marble (m);

$g$ : gravitational constant  $\approx 9.81$ .

The kinematic viscosity is inversely proportional to the density of the fluid and is given by equation 3.

$$v = \frac{\eta}{\rho_{\text{fluid}}} \quad (3)$$

Where;

$v$ : kinematic viscosity in m<sup>2</sup>/s;

$\eta$ : dynamic viscosity in Pa. s;

$\rho_{\text{fluid}}$ : fluid density

The cetane number of the grease was determined using the ASTM D 613 standard as described by Molla and Nigus [16] and the heating value was determined using the ISO 1523 standard. The chemical properties including the acid number, iodine number, and saponification number of crude grease of the WRL were determined respectively by titration with potassium hydroxide (ASTM D-664), NFT60-204 and the NF T60-206 standard. The sulphated ash content was determined using the EN ISO 3987 standard, while the phosphorus content was determined using the spectrophotometric assay. The sulfur content was determined using the EN ISO 20846 standard.

### 2.4 Biodiesel Production

The biodiesel production was accomplished using a two-step transesterification process, namely acid-catalyzed esterification and

alkaline-catalyzed transesterification as describe by Hui et al. [9]. The process of bioconversion of WRL grease to biodiesel is shown in Fig. 1. The methyl ester yield (MEy) was calculated using the following formula:

$$ME_y = \frac{W_{me}}{W_o} \times 100 \quad (4)$$

Where  $ME_y$  is the methyl ester yield,  $W_{me}$  the mass of methyl ester in g, and  $W_o$  is the mass of oil in g.

### 2.5 Fuel Properties of Biodiesel

To verify if the biodiesel produced respected the American and European norms, the cetane number was determined according to ASTM D-613 standard while the calorific value was determined using the ISO 1523 standard. The density of biodiesel was determined by the hydrometer method (ASTM D-1298) and kinematic viscosity was deduced from the density

through equation 5 as describe by Lakshmi et al. [17].

$$u = \frac{929,59 - \rho}{15,77} \quad (5)$$

The flash point (fp) of biodiesel was determined with the equation 6 describe by Lakshmi et al. [17].

$$fp = 12,36u + 176,3 \quad (6)$$

Where:

- $u$  = kinematic Viscosity à 40°C ( $mm^2s^{-1}$ )
- $fp$  = flash point (°C)
- $\rho$  = density ( $kg\ m^{-3}$ )

The chemical proprieties including the acid number, iodine number, sulphated ash content, phosphorus content and sulphur content of WRL biodiesel were determined with the same methodologies used in section 2.3 for crude grease analysis.

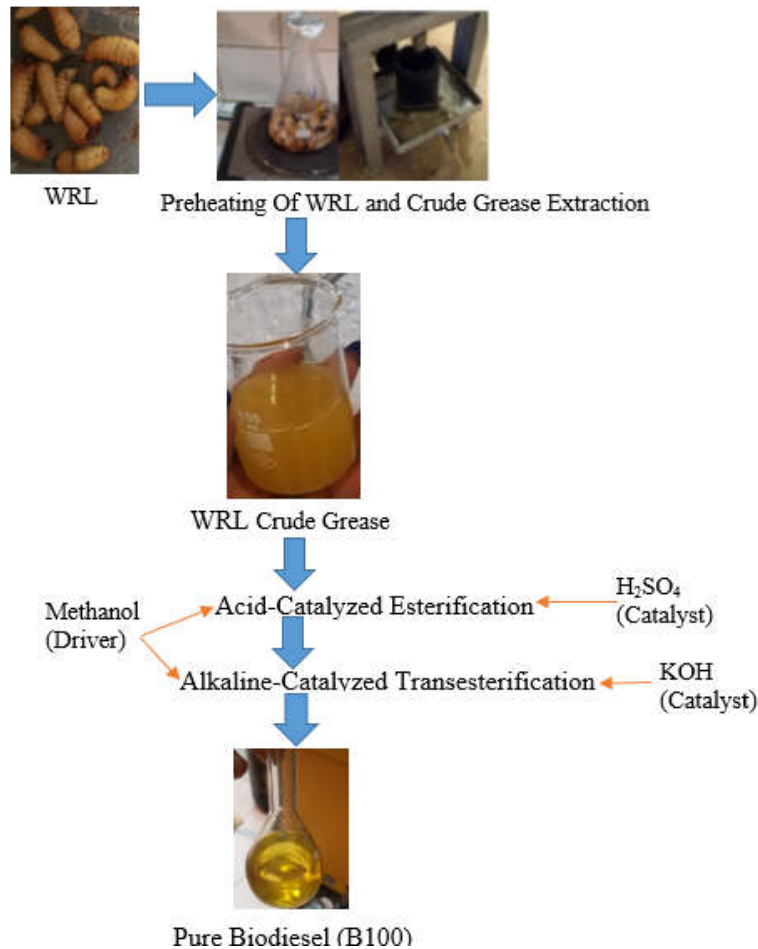


Fig. 1. Technical process of bioconversion of white raphia larvae grease to biodiesel

## 2.6 Chemical Composition of Defatted *R. phoenicis* Larvae Meal

The chemical analysis of WRL defatted was carried out to find out if the pressed cake was suitable for human and animal consumption. The moisture content, dry matter, organic matter and ash content of WRL defatted were analyzed using the procedures described by the Association of Official Analytical Chemists (AOAC), [18]. The Nitrogen content was determined using the Kjeldhal analytical procedure. The crude protein content was calculated by multiplying the nitrogen content by a factor of 6.25 (crude protein = nitrogen content  $\times$  6.25). The fat content was determined using the Soxhlet method.

The iron, calcium, magnesium, sodium, phosphorus and potassium contents in the larval residues were determined with atomic absorption spectroscopy (AAS). For this purpose, 1g of each sample was calcinated in an oven at 450°C for 2 hours. Then, these samples were digested with 10 ml of nitric acid of 1N concentration for 30 minutes and then cooled and filtered with filter paper in the 50 ml volumetric flasks. The volume was completed with distilled water up to the mark and the samples were read directly by atomic absorption spectrometer at 430nm. Each experiment was performed in triplicate and the results were reported as the mean  $\pm$  standard deviation.

## 3. RESULTS AND DISCUSSION

### 3.1 Extraction Parameters of Insect Biomass

Table 1 present the parameters obtained after breeding and extraction of crude grease from WRL. The extraction yield of WRL was about 23%. This result is close to that of [19] who obtained a lipid extraction yield of  $24.3 \pm 1.2\%$  and  $24.3 \pm 1.2\%$  after extraction by soxhlet respectively on the insects *A. domesticus* and *T. Molitor*. In addition, the mechanical extraction method has the advantage of keeping the extraction residue without chemical contaminants, which can be used as enriched protein source for food or feed [20].

### 3.2 Physical and Chemical Properties of WRL Crude Grease

The physico-chemical properties of WRL crude grease were comparable to those of rapeseed oil

(Table 2). However, the kinematic viscosity of WRL at 40°C was lower than that of rapeseed oil at 37.8°C. This result can be explained by the fact that WRL crude grease has a percentage of saturated fatty acid (95.77%) higher than rapeseed oil (4.02%) [13]. Crude grease from WRL has a lighter density compared to crude oil from rapeseed. It equally has a higher calorific value and a lower iodine value as well as a lower saponification value than rapeseed crude oil. Crude grease from WRL is therefore a better feed stock for biodiesel production.

**Table 1. Conversion parameter of insect biomass into free fatty acid**

Characteristics	Values
Larvae weight (g)	1376.28
Grease mass (g)	300
Grease volume (ml)	334
Extraction yield (%)	22.80

The viscosity obtained in this study was within the suitable range (13-17 mm<sup>2</sup>/s) proposed by Blin et al. [22] for stationary engines. Table 2 shows that the cetane number of WRL grease (105) was higher than that of rapeseed oil (39). This result confirm the fact that vegetable oil cetane number is lower than animal grease [23]. The high cetane number of WRL recorded in this study might lead to high auto ignition capacity of oil subjected to compression and short ignition delay of fuel during injection [24]. The acid value of WRL crude grease was higher than that for rapeseed crude oil. This might be due to the high concentration of saturated fatty acid of WRL [8]. In fact, the more saturated the oil is, the lower its iodine value, and the more it is favorable for good combustion (short evaporation time, short ignition time, fewer deposits) [25].

### 3.3 Physical and Chemical Properties of WRL Biodiesel

The comparison of WRL biodiesel with European biodiesel standard (EN14214), American biodiesel standard (ASTM D6751) and biodiesels derived from black soldier fly (BSF) larvae grease and rapeseed oil are shown in Table 3. Generally, the WRL biodiesel properties meets with the fuel specifications of EN14214 and ASTM D6751 which include density (882 kg/m<sup>3</sup>), viscosity (3.017), sulphur content ( $23 \pm 0.2$  ppm) and iodine value ( $100.42 \pm 0.03$ ).

**Table 2. Comparison of physical and chemical properties of WRL crude grease and rapeseed oil**

Fuel properties	WRL grease Values	Rapeseed crude oil <sup>e</sup>
Kinematic viscosity (mm <sup>2</sup> /s)	17.43 <sup>a</sup>	35- 37 <sup>b</sup>
Density at 20°C (kg/ m <sup>3</sup> )	898.3	912
Cetane number	105.11	41
Heating value (MJ/kg)	45.54	39.7
Acid value (mg/g)	5.81 ±0.01	1.4 <sup>c</sup>
Saponification value (mg/g)	81.34±0.02	171.90
Iodine value (g/100g)	36.83 ± 0.04	105

<sup>a</sup> kinematic viscosity at 40°C, <sup>b</sup> kinematic viscosity at 37,8°C, <sup>c</sup> [21], The data for rapeseed oil was from Blin et al., [22]

**Table 3. Physical properties of WRL biodiesel compared to the European biodiesel standard (EN14214), the American Society of Testing materials, ASTM D6751, standard and BSF larvae biodiesel**

Characteristics	WRL biodiesel	BSF biodiesel <sup>a</sup>	Rapeseed biodiesel <sup>b</sup>	EN14214 standard		ASTM D6751 standard	
				Min	Max	Min	Max
Density 15°C (kg/m <sup>3</sup> )	882	885	880 ,830	860	900		
Methyl esters yields (%)	85						
Viscosity 40°C (Mm <sup>2</sup> /s)	3.017	5,8	4.60 6.35,	3.5	5.0	1.9	6.0
Flash point (°C)	213.599	120	105	120	/	130	/
Cetane number	56.14	53	45	48	60	47	/
Acid value (mg/g)	1.03±0.02	1.1	0.11		<0.8	0.5	
Water content (% vol)	1	0.03	0.03		<0.03	0.05	
Phosphorus content (ppm)	2700 ±0.5	/	/		10	10	
Sulphur content (ppm)	23 ± 0.2	/	<100		100	500	
Iodine value (g/100g)	100.42 ±0,03		118		/	120	

<sup>b</sup> The data for rapeseed oil was from [21] and <sup>a</sup> BSF from [8]

Flash point and cetane number play an important role respectively in fuel stability in storage and good self-ignition ability [26]. The result of the present study shows that, flash point (213.599°C) and cetane number (56.14) of WRL biodiesel meets with the biodiesel standards (ASTM and EN) and are slightly higher than that of BSF larvae and rapeseed. So, this high cetane number and flash point are suitable for a new biodiesel feedstock. Another biodiesel property that plays an important role in the deposits of gums that can block the injectors is phosphorus content. According to the result of the present study, phosphorus content is higher than the European and American standards this might be due to the high phosphorus content of the WRL (6850 ppm) [13]. It is therefore recommended that this phosphorus may be reduced through the process of degumming of fat before transesterification. The iodine value of the

biodiesel analyzed is lower than that of rapeseed oil but met the American standard. Nevertheless, this is an asset for this biodiesel because the lower the iodine value is, the lower the ability of biodiesel to rancid and the better is its combustion [26].

### 3.4 Nutritive Value of Defatted WRL Meal

The nutritive value of defatted extraction residue of WRL is presented in the Table 4. It can be seen that the protein content of WRL residues is higher as compared to that of soybean meal and fishmeal which are the most used protein source in animal nutrition. This suggests that, WRL meal can be used as source of proteins for food and feed. This presents an added advantage over other biodiesel feedstock like *Jatropha* and castor oil which produce poisonous cakes at the end of fuel production.

**Table 4. Nutritive value of *R. phoenicis* larvae meal**

Characteristics	<i>R. phoenicis</i> defatted larval meal	Fish meal <sup>a</sup>	Soybean cake <sup>b</sup>
Dry matter (%)	95.15	92	88.09
Ash (%)	3.42	6.5	7.13
Crude protein (%)	66.76	62.9	46.80
Fat (%)	10.22		1.98
P (ppm)	10825	5.8	7300
Azote (ppm)	7172		-
NA (ppm)	579	0.4	200
Ca (ppm)	260	3.2	3400
Mg (ppm)	12.15	2.5	3300

<sup>a</sup> (Banaszkiewicz, [27]) et <sup>b</sup> (Rabia et al. [28])

#### 4. CONCLUSIONS

In this study we investigated and identified a new feedstock for biodiesel production. *Rhynchophorus phoenicis* crude grease fuel properties revealed that, the density and cetane number were (932.20 kg/m<sup>3</sup> and 105.11 respectively). The calorific value and acid value were relatively higher than those of rapeseed crude oil. WRL crude grease can be used as fuel in bi-fuelled, stationary engine and indirect injection engine. The density of the biodiesel at 15°C was 882 kg / m<sup>3</sup>, the viscosity at 40°C (3.017 mm<sup>2</sup>/s), the flash point (213.6°C) and the cetane number (56.14) were quite comparable with those of EN 14214 and ASTM standard. The defatted extraction exhibited high protein (66.76%) and mineral which make it suitable for animal and human feed protein supplement. Based on this result, it was concluded that crude grease and biodiesel have great potential to satisfy the increasing demands for liquid fuels, particularly in developing country.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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