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Effect of Feeding Substrates on the Oil Quality of *Rhynchophorus pheonicis* and *Orycete rhinoceros* Larvae

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ABSTRACT

This study evaluated the physicochemical properties of oils extracted from larvae of Rhynchophorus phoenicis and Orycete rhinoceros reared on palm pith (PP) and sawdust (SD) substrates. Four treatment groups were analyzed: Large larvae fed on palm pith (PPLL), large larvae fed on saw dust (SDLL), small larvae fed on saw dust (SDSL), and small larvae fed on palm pith (PPSL). Standard analytical methods were used to determine free fatty acid (FFA), acid value, iodine value, and peroxide value. Results showed significant differences ($p < 0.05$) among samples for most parameters. FFA values ranged from 1.66 ± 0.075% in PPSL to 6.51 ± 0.077% in SDLL, indicating varying levels of hydrolytic degradation. Acid values ranged between 0.27 ± 0.048 and 1.16 ± 0.009 mg KOH/g, with SDLL recording the highest value. Iodine values ranged from 1.20 ± 0.067 to 3.256 ± 0.049 g I_2/100 g, suggesting low to moderate unsaturation of the oils. Peroxide values varied significantly from 45.00 ± 4.082 to 79.67 ± 0.47 meq/kg, reflecting differences in oxidative stability. Oils from larvae reared on palm pith generally exhibited better quality indices, especially lower FFA and acid values, compared with some sawdust fed samples. The increasing interest in edible insects as sustainable lipid sources has created the need to assess the quality and industrial suitability of insect oils. The findings demonstrate that rearing substrate significantly influences the quality characteristics of insect oils. These oils may have potential applications in food, feed, cosmetics, and biodiesel industries, provided proper refining and storage measures are implemented. The study supports the use of agro waste in insect farming for sustainable lipid production.

Key words: *Rhynchophorus phoenicis, Oryctes rhinoceros,* Oil quality, Palm pith, Sawdust.

INTRODUCTION

The growing global population and increasing demand for sustainable food resources have intensified the search for alternative sources of nutrients and industrial raw materials. Conventional livestock production is often associated with high feed costs, land use, and environmental burdens. As a result, edible insects have gained considerable attention as promising alternatives due to their high nutritional value, rapid growth rate, and ability to convert organic waste into valuable biomass (Food and Agriculture Organization, 2013). Insects are rich in proteins, lipids, vitamins, and minerals, making them important candidates for food, feed, and industrial applications. Among edible insects, *Rhynchophorus phoenicis* and *Orycete rhinoceros* larvae are widely consumed in many African and Asian communities. These larvae are particularly valued for their high fat content, which can be extracted as oil for culinary, cosmetic, pharmaceutical, and biofuel uses. Previous studies have shown that insect oils contain appreciable levels of fatty acids comparable to some plant and animal fats (Rumpold and Schlüter, 2013).

However, the quality and composition of these oils depend on species, developmental stage, environmental conditions, and feeding substrates. Feeding substrate plays a critical role in determining the nutritional composition of insect larvae. Agro-industrial residues

such as palm pith and sawdust are abundant and inexpensive materials that can serve as rearing substrates. Palm pith, derived from decomposing palm trunks, is naturally rich in organic nutrients and moisture, while sawdust is a lignocellulosic by product generated in wood processing industries. Utilizing these substrates for insect production can reduce environmental waste and support circular economy systems. The physicochemical quality of extracted oil is commonly assessed using parameters such as free fatty acid (FFA), acid value, iodine value, and peroxide value. Free fatty acid and acid value indicate the extent of hydrolysis and deterioration, while iodine value reflects the degree of unsaturation of fatty acids. Peroxide value measures oxidative rancidity and storage stability of oils (Choe and Min, 2006).

These parameters are essential for determining the suitability of oils for food and industrial purposes. Although many studies have investigated the protein value of edible insects, limited information exists on the effect of rearing substrates on the oil quality of *Rhynchophorus phoenicis* and *Orycete rhinoceros* larvae. Therefore, this study aimed to evaluate the physicochemical properties of oils extracted from these larvae raised on palm pith and sawdust. The findings will contribute to the development of sustainable insect oil resources and promote the utilization of agro residues in insect farming systems.

MATERIALS AND METHODS

The study was conducted at the Bioresources Development Centre Odi, Bayelsa State, University of Africa, Toru-Orua, Bayelsa State Nigeria. The area is characterized by a tropical climate favorable for insect growth and development, with relatively high temperature and humidity and Central research laboratory and Diagnostics Ilorin Kwara State

Collection of Larvae

Larvae of *Rhynchophorus phoenicis* and *Orycete rhinoceros* were collected from naturally infested palm trees and decomposing palm trunks in Odi Community of Kolokuma Opokuma local Government area. The larvae were identified morphologically and sorted according to species. Healthy and active larvae were selected for the experiment.
Two feed diet were used, the palm pith (PP) and sawdust (SD). Palm pith was obtained from decaying palm trunks and chopped into smaller pieces. Sawdust was collected from untreated wood processing sites near BIODEC Odi, air dried and moistened with clean water to provide suitable humidity for larval growth.

Experimental Design

The experiment consisted of two treatment groups based on insect species and feeding diets
SDSL -*Rhynchophorus phoenicis* larvae raised on sawdust
PPSL- *Rhynchophorus phoenicis* larvae raised on palm pith
PPLL - *Orycete rhinoceros* larvae raised on palm pith
SDLL - *Orycete rhinoceros* larvae raised on sawdust

Exactly 40 Palm weevil larvae each *Rhynchophorus phoenicis and Orycete rhinoceros* larvae and palm pith were collected in sterile plastic containers, in June, 2023 from Odi community, Kolokuma/Okpokuma Local Government Area and Toru-Orua community, Sagbama Local Government Area, while Saw dust was collected from sawmill in Odi community, Bayelsa State. *Rychophorus phoenicis larvae and Orycete rhinoceros* larvae were acclimatized for one week at the Bayelsa Suya domestication Lab in Bioresources Development Centre Odi in Bayelsa State. The lab was properly ventilated by opening all the windows. At room temperature all the larvae were fed with palm pith. The various media were prepared and divided into three transparent plastics. Three (3) each of

Rhychophorus phoenicis and Orycete rhinoceros larvae were transfer into a well labeled transparent plastic containers and bred for three weeks. The media were rehydrated with 12mL of water using the pressure spray bottle pump at alternating days. Changing of the media and weighing of the larvae every week for three weeks was carried out.

Sample Preparation

At the end of the rearing period, larvae were harvested, washed thoroughly with distilled water to remove adhering materials. The larvae were put in the freezer compartment of a Thermocool fridge for 15mins to inactivate the larvae.

Lipid content determination

Cold extracting method of lipid from larvae (*Rynochophorus phoenicis and Oryctes rhinoceros*). Exactly 100mL beakers capacity was washed and sterilized. They were set in triplicate and well labeled properly. Exactly 3g of the *Rynchophorus phoenicis* and *Oryctes rhinoceros* larvae were weighed and placed inside the labeled beaker accordingly, in which 30mL of N-hexane was measured using measuring cylinder and dispensed into each of the beakers covered with foil paper and placed on orbital shaker (CLETGCH KJ-20IBD) at 50rmp for 72 hours. After 72 hours a cleaned and well labelled beaker was all weighed and recorded accordingly. The various N-hexane was decanted from the sample mixture. They were left at room temperature for the N-hexane to dry up.

Determination of Physicochemical Properties of *Rynchophorus pheonicis* and *Orycetes rhinoceros*

The extracted oils were analyzed for the following parameters using standard analytical procedures of the Association of Official Analytical Chemists:

Free Fatty Acid (FFA, %)

Into a 250ml conical flask, 0.5g of the extracted *Ryhnochophorus pheonicis and Oryctes rhinoceros* sample was weighed into it using a digital electronic weighing balance into it. To it was added 10ml of premixed chloroform/methanol mixture 1;1. The content was shook properly to mix up. Exactly two (2) drops of phenolphthaleins was added (0.2ml).The content was titrated with 0.1M sodium hydroxide solution to a light pink endpoint which persisted for 1minutes.

The blank was determined in the same way in the absent of the sample and the burette reading recorded and free fatty acid calculated according to the formula.
Calculation for free fatty acid

Free fatty acid	=	Titrant value x M x 281
M	=	the molarity of the base (NaOH
282	=	constant

Acid Value (mg KOH/g)

Exactly 0.5g of the *Ryhnochophorus pheonicis and Oryctes rhinoceros* sample homogenate was weighed in a cleaned conical flask and 10mL of ethanol| petroleum ether (1:1) was added. Phenothalien indicator 0.2mL was added. The solution was titrated with 0.1M KOH solution to a pink coloration which persisted for 30 seconds. A blank was titrated containing all the reagents without the sample.

Calculation Acid value: VK x N x 5.61/W
VK=Potassium hydroxide titrate used (ml)
W=weight of fatty acid being examinied (g)
N=Normality or molarity of KOH

Iodine Value (g I_2/100 g)

Into a conical flask of 250ml capacity was weighed 0.1g of *Ryhnochophorus pheonicis and Orycete rhinoceros* sample homogenate and in it was added 40ml of wijs solution and shook properly to mix (chloroform and methanol ratio 1:1).The mixture was allowed to stand for 30minutes in the dark after which 3.0ml of saturated potassium iodine 10% (W|V) was added, followed by 20ml of distilled water. The mixture was titrated with 0.1N sodiumthiosulphate in a burette to a clear solution. The blank was done using all the reagents involved without the sample. Iodine value calculated.

Calculation for Iodine value: (B – S) x N x12.69/W
Where B = is the blank titre value
S = is the sample titre value
N=is the normality titrant
W = weight of the sample

Peroxide Value (meq/kg)

In a 250mL capacity conical flask, 0.5g of the *Ryhnochophorus pheonicis and Oryctes rhinoceros* sample homogenate was weighed using electronic digital weighing balance into it. Exactly 15mL acetic acid /chloroform at ratio 3:2) was added. The solution was thoroughly shaken and 0.25mL /250mL of potassium iodine (KI) was added and shaken. This was allowed to stand for 1minute to develop color. Into it was added 15mL of distilled water, followed by 250mL/ 0.25mL of 1% solution. The mixture was titrated with 0.1N sodium thiosulphate to a milky endpoint. The blank was conducted in a similar way without the sample.
Calculation for Perioxide value: (S- B x 0.1x 1000)/W
Where B= the blank titre value
S=the sample titre value
W= sample weight
1000 = is a constant

Statistical Analysis

All analyses were conducted in triplicate, and results were expressed as mean ± standard deviation. Data were subjected to one way ANOVA, and means were separated using Duncan's Multiple Range Test at a significance level of $p < 0.05$.

RESULTS

Table 4.1: Physicochemical Properties of Oil Samples

Sample	FFA (%)	AV (mg/KOHg)	IV (gI_2/100g)	PV (meq/kg)
PPLL	3.36 ± 0.01^{c}	0.88 ± 0.053^{b}	3.256 ± 0.049^{a}	79.67 ± 0.47^{c}
SDLL	6.51 ± 0.077^{d}	1.16 ± 0.009^{c}		70.00 ± 0.000^{b}
SDSL	2.23 ± 0.003^{b}	0.27 ± 0.048^{a}	1.20 ± 0.067^{a}	79.00 ± 0.816^{c}
PPSL	1.66 ± 0.075^{a}	0.28 ± 0.001^{a}	1.27 ± 0.000^{a}	45.00 ± 4.082^{a}
			2.19 ± 0.038^{a}	

Values are mean ± SEM. Means with different superscripts within the same column are significantly different ($p < 0.05$).

CODES
PPLL-large larvae fed on palm pith
SDLL- large larvae fed on saw dust
SDSL-small larvae fed on saw dust
PPSL-small larvae fed on palm pith
FFA- free fatty acid
AV- acid value

DISCUSSION

The physicochemical properties of oils extracted from insect larvae are important indicators of their nutritional quality, shelf stability, and industrial applicability. In this study, significant differences ($p < 0.05$) were observed among oil samples, suggesting that both insect species and rearing substrates influenced lipid composition. Free fatty acid (FFA) values were highest in SDLL (6.51%) and lowest in PPSL (1.66%). High FFA values are usually associated with lipase activity, hydrolysis, or poor storage conditions, and they may reduce oil quality and palatability. Lower FFA values in PPSL indicate better oil stability and freshness. Similar observations have been reported in edible insect oils where processing and feed substrate affected lipid hydrolysis (Womeni *et al*., 2009; Rumpold and Schlüter, 2013).Acid value followed a similar trend, with SDLL having the highest value (1.16 mg KOH/g). Acid value reflects the amount of free fatty acids present in the oil and is commonly used to assess deterioration. Oils with lower acid values are preferred for consumption and biodiesel processing because they require less refining (FAO, 2019). The relatively low acid values observed in PPLL, SDSL, and PPSL indicate acceptable oil quality. Iodine value measures the degree of unsaturation in oils.

The highest iodine value recorded in PPLL (3.256 g I_2/100 g) suggests a greater proportion of unsaturated fatty acids compared with the other samples. Unsaturated fats are nutritionally desirable due to their beneficial effects on cardiovascular health, although they are more prone to oxidation (Bukkens, 2005). The generally low iodine values in this study may indicate predominance of saturated and monounsaturated fatty acids. Peroxide value is an indicator of primary oxidation products formed during rancidity. PPLL and SDSL showed higher peroxide values, while PPSL had the lowest value. Lower peroxide values imply better oxidative stability and longer shelf life. The elevated peroxide values in some samples may be linked to exposure to heat, oxygen, or inherent fatty acid composition during extraction and storage (Choe and Min, 2006).

Overall, larvae reared on palm pith appeared to produce oils with more desirable quality characteristics than some sawdust-fed groups. This may be due to the richer nutrient composition of palm pith, which can influence lipid metabolism and fatty acid deposition in larvae. These findings support previous studies showing that insect diet significantly affects body fat quality and composition (Oonincx and van derPoel, 2011).

CONCLUSION

This analysis revealed that the oil quality of *Rynchophorus pheonicis* and *Oycete rhinoceros* are significantly influenced by feed, larvae type, and moisture exposure. Samples with low FFA, acid value, and peroxide value demonstrated better stability and longer shelf life, while those with high values showed rapid deterioration which is PPLL and SDLL.

RECOMMENDATIONS

Recommendations on Public Consumption and Edibility of the Insects

Based on the findings of this study and the nutritional potential of edible insects, the following recommendations are made regarding the consumption of *Rhynchophorus phoenicis* and *Orycete rhinoceros:*

1. These larvae can serve as valuable alternative sources of edible oil, protein, and energy when properly harvested, processed, and cooked under hygienic conditions. Public awareness should be increased on their nutritional benefits.

2. The insects should be thoroughly cleaned and adequately cooked or processed before consumption to reduce microbial contamination and improve safety. Raw consumption is not recommended.

3. Consumers are encouraged to obtain insect products from hygienic and controlled farming systems rather than indiscriminate wild harvesting, which may expose them to contaminants or spoilage.

4. The larvae may be incorporated into local diets as supplementary protein and fat sources, especially in communities facing food insecurity or high costs of conventional animal protein.

5. Government agencies, researchers, and food industries should explore the development of insect-based products such as cooking oil blends, snacks, flour, and animal feed ingredients.

6. Health authorities should educate the public on safe edible insect handling, storage, and preparation methods to improve consumer acceptance and reduce misconceptions.

7. More studies should be conducted on long term safety, nutrient composition, and acceptable processing methods, while regulatory bodies like National Agency Food and Drug Administration Control (NAFDAC), Nutrition Society of Nigeria (NSN) and Nigeria Institute of Food Science and Technology (NIFST) should establish standards for edible insect products.

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