The Study of the Physical Indices and Characterization of Oil Extract from Rhynchophorus Ferrugineus Larva (Raffia Palm Maggot)

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Abstract: This paper carried out experimental study through extraction and characterization of oil extract from *Rhynchophorus ferrugineus* larvae. Normal hexane was used as solvent for the Soxhlet method of extraction which gives the highest percentage oil yield of 24.6% of all the other methods (Maceration method 18.2%, Cold Extraction Method 10.6%). The physicochemical analysis revealed that the tested paramaters which includes, iodine value, specific gravity, refractive index, saponification value, cholesterol value, acid value, peroxide value, free fatty acid value, viscosity etc falls within the CODEX acceptable limit of edible vegetable oil. The unsaturated nature of the oil was revealed by the fatty acid profile test using gas chromatography because of the presence of palmitoleate which is an ester of palmitoleic acid, an omega 7 mono unsaturated fatty acid and also by the iodine value (128.1892mg/g) which is a measure of the degree of unsaturation in edible oil, which falls within the range of class of oil known as semidrying oil, suggesting that the oil contains some proportion of unsaturated double bonds thereby, the oil is healthy for consumption. Elemental Analysis of the oil using Atomic Absorption Spectrophotometer indicated the presence of metals such as calcium, zinc, magnesium, manganese, iron etc in the oil. Determination of Vitamins ascertained the presence of some vitamins such as Vitamin D, B_1, E, C and A.

Keywords: Cold extraction, Oil extract, Palmitoleic acid, Rhynchophorus ferrugineus.

I. Introduction

Fats and oils are water insoluble substances of plant and animal origins, which consist predominantly of long chain fatty acid esters of glycerol and are known as triglycerides. The quest for fats and oils has continued to receive attention, because of high consumption of fat. However, it has been observed, that the amount of saturated fat consumed, is the primary factor to be taken into consideration, because it increases the bad LDL cholesterol in the blood stream, which leads to coronary heart disease, clogged arteries and stroke (John, 2008).

It has also been realized that poly-unsaturated fatty acids are not beneficial to health as previously believed. Mono-unsaturated fatty acid is more beneficial than poly-unsaturated fatty acid (Primorac et al, 2000).

A. Background of the Study

Rhynchophorus ferrugineus larva, commonly known as edible maggot, is a great source of protein, carbohydrate and widely believed to be rich in fatty acid (EPPO, 2008). It also contains phosphorus, calcium, zinc etc (Murphy and Briscoe, 1999). The larvae contain a great amount of fibre that could serve as a great source of diet roughages. This study seeks to isolate, identify and characterize the physical and chemical compositions of the oil. Proximate and Spectroscopic analysis will also be carried out on the residue obtained after the extraction of the oil.

B. Statement of the Problem

The consumption of *Rhynchophorus ferrugineus* (larva) has continued to attract attention as a food delicacy over the years in some areas of the world. But the preparation for consumption has relied solely on roasting and frying after they are boiled with little salt and water. Some indigenous people consumed the raw larva without considering the health implications or otherwise and the nature of the oil contained in the larva. Systematic efforts have been made to carry out proximate and elemental analysis of the oil towards establishing the chemical and physical properties, including the assessment of the cholesterol composition level, iodine value, etc according to Onuchukwu, et al, 2010. Fats and oils are classified into: drying oil, semi-drying oil and non drying oil. The volatility of the oil, odour, colour, specific gravity etc., are issues thrown up for elucidation and parameters sought towards establishing the quality of the oil extract.

C. Research Question

In the frame work, the study will seek empirical answers to these specific research questions:

- What is the nature of fatty acid and sterol composition of the oil and the mineral composition of the oil extract?
- What is the process of extraction and separation of the fatty acid components in the raffia larva?
- What are the processes of identification and characterization of the oil extract and assessing the yield of fat and oil, carbohydrate and protein contents of raffia larva?

D. Objective of the Study

The specific objective of this research work includes the following:

- To extract oil from (*Rhynchophorus ferrugineus*) larva.
- To characterize the oil for its chemical composition, such as determination of acid value, iodine value, free fatty acid, saponification value, nature of oil with respect to saturation or otherwise, etc.
- To determine the mineral composition of the oil.
- To determine the quality of the oil with respect to yield per larva, protein, carbohydrate, etc.
- To elucidate and establish the type and quantity of the fatty acids and sterol present.
- To assess fairly the nutritional, industrial and medicinal values of the oil.

E. Scope of Study

- Sourcing the Specimen: The samples used were ideally fresh raffia maggot which was sourced from palm wine tappers along the river basins at Oraifite and Akwu-ukwu towns in Anambra State and stored in ice cooler at a temperature between 0^{o}C and 5^{o}C.
- Extraction and Separation: Soxhlet extraction, Maceration and Cold Extractods was applied in the extraction of the oil and the one with high percentage oil yield noted. It involves grinding the washed and dried raffia maggot with electric grinder (Otedoh, 1974).
- Separation and Purification: The oil extract obtained from the raffia maggot was subjected to gas chromatography.
- Identification and Characterization: The physical and chemical analysis of an oil parameter was determined. The fatty acid composition and sterol composition of the oil were identified and characterized using gas chromatography and mass spectrometer. These spectral measurements were applied to obtain information of the fatty acid compositions and sterol composition of the oil (AOAC, 1995).
- Physiochemical Analysis: The physiochemical analysis of the oil extracted from the raffia maggot was obtained by conducting the following qualitative test such as iodine value, saponification value, flash point etc.
- Elemental Analysis: The elemental analysis of the residue of raffia maggot was carried out using Atomic Absorption Spectroscopy (AAS). The following mineral elements that were determined are Calcium, Sodium, Iron, Magnesium, Manganese, etc. (Mc Dowell, 1992).
- The Fibre Content: The amount of fibre content of the larvae was investigated through proximate analysis alongside with the moisture, fat, protein ash, available and unavailable carbohydrate, etc.
- Determination of Vitamins: Test for various vitamins are run to identify the type of vitamins present in the oil extract (Onwuka, 2005).

F. Raffia Palm Larva (Rhynchophorus Ferrugineus)

Larva: The larva of the raffia palm weevil "*Rhynchophorus ferrugineus"* (grub) as shown in Fig. 1, has popularly been known as "ERURU" for Igbo's in the Eastern Nigeria. It is a species of maggot (grub) though a giant maggot and are known by different ethnic groups in Nigeria, Africa, far Asia and some part of Europe. The larva is a great source of protein and widely believed to be rich in fatty acid. It is popular where raffia palm trees are found. This extends to the inhabitants of Amazon forest, Malaysia, Indonesia, New Guinea and more. The full grown larva is conical in shape and is a legless fleshy grub, while the newly hatched larva is yellowish white in colour, with a hard brown head. The average length of the full grown larva is 50mm and the width is 20mm. Larva feed within the soft tissues of the meristem or leaf bases creating frass filled mines, enlarging and penetrating deep within the upper trunk areas as the larva matures. Grown larva constructs a pupa chamber or cocoon made of coarse palm fibres in which they pupate and occupy for approximately three to four weeks. The cocoons are situated within the damaged tissue of the palm. The mouth of the grub is well developed and strongly chitinized, which enables the grub to burrow into the trunk of palm trees and raffia palms (Faleiro et al, 1998).

Fig. 1. Rhynchophorus ferrugineus larva

G. Life Cycle of Rhynchophorus Ferrugineus Larva

Rhynchophorus ferrugineus (grub) undergoes complete metarmophosis which consists of (egg, larva, pupa and adult) asd shown in Fig. 2. The stages are spent inside the moist-trunk environment (Bokhari and Abuzuhira, 1992). Females use their long beak or rostrum, to chew a hole into palm tissue. Eggs are then laid into this hole. Eggs may be laid in wounds, cracks and cravices in the trunk, from the collar region near the roots, up to the base of frond petioles and axils near the crown of the palm. Female can lay between 58-500 eggs which hatch within 5 days into legless grub that bore into the interior of the palms, moving by peristaltic muscular contractions of the body and feed on the soft succulent tissues, discarding all fibrous material. The tunnels larva form as they feed, fill with frass (excrement and chewed fibres that have a highly distinctive odour) and plant sap. Larva pass through 3-7 stages that may last between 1-3 months before the pupa stage is reached. Larva pupates inside cocoons in the palm trunk. The pupa elongates oval cylindrical cocoons made of fibrous strands. At the end of the pupate period which lasts between 14-21 days, the adult weevil emerges (Hallet et al, 2004).

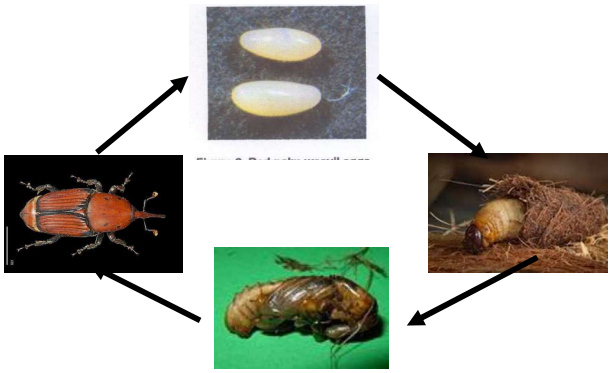

Fig. 2. The life cycle of *Rhynchophorus ferrugineus* (larva)

The total life cycle takes about 4 months (Hallet et al, 2004).

II. Materials and Methods

A. Sample Collection

The (*Rhynchophorus ferrugineus*) larvae samples were sourced from Oraifite town in Anambra State Nigeria. The larvae were 69 in number; fully grown larvae of different sizes. Their mouth parts are well developed and strongly chitinized as shown below:

Fig. 3. The samples (*Rhynchophorus ferrugineus* (larvae))

These larvae have their habitat in dead raffia palm or spent raffia palm from tapping or where the process of wine tapping is accomplished. These raffia trees will be cut and their stem will be pierced through the holes, created inside the palm by these maggots to be followed gently until their habitat is reached. They were collected into a container.

B. Identification and Authentication of the Sample

The samples were identified and authenticated at the Entomology Laboratory of the Department of Biological Science, Anambra State University Uli, Nigeria.

C. Taxonomy

Kingdom:	*Animalia*
Phylum:	*Arthropoda*
Order:	*Coleoptera*
Class:	*hexapoda(insecta)*
Common Name:	Raffia Palm Weevil
Scientific Name:	*Rhynchophorus ferrugineus*
Family:	*Curclionidae*
Local Name:	Eruru ngwo or akpa ngwo

In some regions of this country, raffia maggots serve as a delicacy.

D. Preparation of Sample

The samples of *Rhynchophorus ferrugineus* were washed with water in order to do away with dirt. Then, the samples were put into a well ventilated container and taken to the laboratory where other processes took place.

E. Determination of Moisture Content

The beaker was washed and dried in the oven. Exactly 5g of the larvae was measured into the beaker. The weight of the beaker and the sample was noted before drying. The larvae were dried to a constant weight in an oven at a temperature of 115^{o}C to remove all the moisture present and the weight was noted. The dried larvae were put into the beaker and reweighed.

Calculation

$$\% \text{ Moisture Content} = \frac{W_1 - W_2}{W} \times \frac{100}{1}$$

Where;

W = Weight of the sample

W_1 = Weight of beaker and sample before drying

W_2 = Weight of beaker and samples after drying

F. Determination of Ash Content

Empty platinum crucible was washed, dried in an oven at a temperature of 105°C and allowed to cool. After cooling, it was weighed and the weight noted (W_1). 5g of wet sample (larva) was weighed into the platinum crucible and weight noted (W_2). The crucible containing the defatted sample was placed in a muffle furnace and heated at 500^0C for 3 hours. The heating was continued until all carbon has been burnt away. The furnace was switched off, and the crucible was taken out and the sample was cooled in a desiccator after burning and weighed.

$$\% \text{ Ash Content} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

W_1 = Weight of the empty platinum crucible

W_2 = Weight of platinum crucible + sample before burning

W_3 = Weight of platinum crucible + ash

W = Weight of sample

Weight of ash only = $W_3 - W_1$

Weight of wet sample = $W_2 - W_1$

G. Determination of Crude Fibre

2g of material was defatted with petroleum ether (if the fat content is more than 10%), boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml of solution. The solution was filtered through linen or several layers of cheese cloth on a fluted funnel, washed with boiling water until the washings are no longer acid. The residue was transferred to a beaker and boiled for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The final residue was filtered through a thin but closed pad of washed and ignited asbestos in a Gooch crucible dried in an electric oven and weighed. Incinerated, cooled and weighed. The loss in weight after incineration x 100 is the percentage of crude fibre.

$$\% \text{ Crude Fibre} = \frac{\text{Weight of Fibre}}{\text{Weight of Sample}} \times \frac{100}{1}$$

$$= \frac{\text{Weight of Crucible + Dried Residue} - \text{Weight of Crucible + Ash}}{\text{Weight of Sample}} \times \frac{100}{1}$$

H. Soxhlet Fat Extraction Method

This method is carried out by continuously extracting a food with non polar organic solvent such as petroleum ether for about 1 hour or more.

$$\% \text{ Crude Fat} = \frac{W_2 - W_1}{W} \times 100$$

Where;

W_1 = Weight of empty flask (g)

W_2 = Weight of flask and extracted fat (g)

W = Weight of sample

I. Determination of Crude Protein

Exactly 1g of sample was weighed into a 30ml kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and shaken. Then 1g of the kjehdal catalyst mixture was added. The mixture was heated cautiously in a digestion rack, until a clear solution is obtained. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling 100ml of distilled water was added to avoid caking and then 50ml of aliquot was transferred to the kjehdal distillation apparatus. A 100ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops of bromocresol blue and 1 drop of methylene blue was placed under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution. The 5ml of 40% sodium hydroxide was added to the digested sample in the apparatus and which it was titrated to pink colour using 0.01M hydrochloric acid.

% Nitrogen = Titre value x 4 x14 x 0.01

Where 14 = atomic number of nitrogen

4 = Dilution Constant

0.01 = Molarity of acid (HCl) used

3.3 = Titre value

Where 6.25 is a constant

% Protein = % Nitrogen x 6.25

J. Determination of Percentage Oil Yield from Different Oil Extraction Methods Used

a. Cold Extraction Method

5g of the ground dry sample was transferred into 1000ml volumetric flask. 200ml of solvent was added to the ground dry sample, which was covered and left for 24 hours. After 24 hours, the oil extract were filtered and the filtrate evaporated. Percentage Oil yield is calculated as follows:

Calculation

$$\% \text{ Oil Yield} = \frac{\text{Weight of Flask + Oil Extract} - \text{Weight of Flask}}{\text{Weight of Sample}} \times \frac{100}{1}$$

b. Maceration Method of Extraction

5g of the ground dry sample was measured into a volumetric flask. 200ml of solvent (water) was added to the ground dry sample, which was covered and boiled for 2 hours, until the oil extract separates from the solvent. The oil was decanted and further heated for the solvent to evaporate. Percentage oil yield was calculated as follows:

$$\% \text{ Oil Yield} = \frac{\text{Weight of Flask + Oil Extract} - \text{Weight of Flask}}{\text{Weight of Sample}} \times \frac{100}{1}$$

c. Soxhlet Extraction Method

5g of the ground dry sample was measured into the extraction thimble, which was closed with a fat – free cotton wool. The thimble was inserted in the Soxhlet extractor. 150ml of normal Hexane was poured into a round bottom flask, the Soxhlet was heated at a temperature of 110°C – 130°C for 6hours. When the solvent was boiling, the vapour rises through the vertical tube into the condenser at the top. The liquid condensate drips into the filter paper thimble in the center which contains the solid sample to be extracted. The oil extract seeps through the pores of the thimble and fills the siphon tube, where it flows back down into the round bottom flask. This was allowed to continue for 30 minutes. The flask containing the oil was then removed from the tube, dried in the oven at 103°C and heated to a constant weight (indicating evaporation of all solvent) and cooled in the desiccators and weighed to determine the amount of oil extracted. Percentage Oil yield is calculated as follows:

$$\% \text{ Oil Yield} = \frac{\text{Weight of Flask + Oil Extract} - \text{Weight of Flask}}{\text{Weight of Sample}} \times \frac{100}{1}$$

d. Determination of P^H

P^H was measured by electrometric method using laboratory p^H Hanna model H1991300. The P^H electrode was rinsed with distilled water and blotted dry. 2g of the oil sample was poured into a clean dry beaker. The p^H electrode was then immersed into the sample and the p^H value was read and recorded.

e. Determination of Refractive Index

Abbe's Refractometer was used in the determination. Few drops of the oil sample was transferred into the glass slide of the refractometer. Water at 30°C was circulated round the glass slide to keep its temperature uniform. Through the eye piece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. The pointer on the scale pointed to the refractive index and the value noted and recorded as refractive index.

f. Appearance of the Oil

The oil was kept inside a container at room temperature for days.

g. Determination of Colour

5ml of the oil sample were poured into a glass cell and stood in the tintometer. One of the two parallel beams inside the instrument passed through the oil sample and the other beam passed through into which the coloured slides were inserted. Both beams of light were brought together into a periscope. Glass slides were put in place until the colour produced by the slides matches the colour of the oil.

h. Determination of Viscosity

A clean, dried Ostwald viscometer with a flow time above 200secs for the fluid to be tested was selected. The sample was filtered through a sintered glass (fine mesh screen) to eliminate dust and other solid material in the liquid sample. The viscosity meter was charged with the sample by inverting the tube's thinner arm into the liquid sample and suction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed into a holder and inserted to a constant temperature bath set at 29°C and allowed approximately 10mins for the sample to come to the bath temperature of 29°C. The suction force was the applied to the thinner arm to draw the sample slightly above the upper timing mark. The afflux time by timing the flow of the sample as it flows freely from the upper timing mark to the lower timing mark was recorded**.**

K. Tests for Unsaturation

a. $KMnO_4$ Test (BDH, Analar Grade 97%)

2 drops of oil sample was dissolved in 2ml of acetone and 2% of $KMnO_4$ was added drop wise. $KMnO_4$ was decolourized showing that the compound is unsaturated.

b. Bromine Test

2 drops of oil sample was added to 2ml of tetrachloromethane, CCl_4 and shaken to dissolve. 2% solution of bromine in CCl_4 was added drop wise while shaking. The colour of the bromine was discharged which indicates that the test compound is unsaturated.

c. Protein Identification

Few drops of Millon's reagent was added to a few cm^3 of grinded raffia palm maggot in a test tube and heated gently. Red colour develops which shows the presence of protein.

d. Determination of Reducing Sugar (Glucose)

10g of the oil sample was weighed and transferred to a 200ml Kohlrausch flask, diluted to volume with distilled water, shaken for 30mins and gravity filtered through Whatman No. 1 filter paper. The filtrate was collected in a clean dry glass. 10ml of Fehling's A solution and 10ml of Fehling's B solution were pipette into 250ml Erlenmeyer flask. 20ml of sample titrate and 10ml of purified water were added to bring total volume of reaction mixture to 50ml. Contents of the flask were mixed by gentle swirling.

2 small glass beads were added to prevent bumping while boiling, with the mouth of the flask covered with a small glass funnel of glass bulb. The flask was placed on a hot plate adjusted to bring the solution to a boil in 3mins and continued boiling for exactly 2mins (total heating of 5mins), cooled quickly to room temperature in an ice bath.

10ml of 30% potassium iodide solution and 10ml of 28% sulphuric acid solution were added and titrated immediately

with standand 0.1M sodium thiosulphate solution, 1ml of starch indicator solution was added while titration continued and the solution carefully agitated continuously until the blue black starch iodine colour disappears.

Two blank determinations were conducted in identical fashion substituting purified water for the sample filtrate. Percentage of reducing sugar is calculated as follows:

Reducing Sugars, % (as dextrose)

$$= \frac{\text{mg Dextrose (from table) x 200ml x 100}}{\text{Sample weight (g) x 20ml x 1000mg/g}}$$

e. Determination of Smoke Point, Flash Point, Cloud Point and Fire Point

10ml of oil was poured into an evaporating dish, a thermometer was suspended at the centre of the dish, ensuring that the bulb dips into the oil without touching the bottom of the dish. The temperature of the oil is raised gradually using a kerosene stove or electric stove.

The temperature at which the oil sample gives off a thin smoke continuously is noted as the smoke point.

The temperature at which the oil starts flashing without supporting combustion is noted as the flash point.

The temperature at which the oil combustion is sustained is called the Fire Point.

The temperature at which solid in the oil begins to form and separtateds from the oil is called the cloud point.

f. Determination of Acid Value

1g of the oil sample was weighed and dissolved with 50ml of ethanol in a conical flask. 2 drops of phenolphthalein indicator were added and titrated to pink end point (which persisted for 15 minutes) with 0.1M potassium hydroxide solution (KOH).

$$\text{Acid Value} = \frac{56.1 \times V \times M}{W}$$

Where;

56.1 = Molecular weight of KOH

V = Volume in ml of standard KOH used

M = Molarity of KOH (0.1M) or exact concentration in KOH used

W = Weight in gram of the test sample

g. Saponification Value Determination

2 grams of oil samples were weighed into a conical flask and 25 ml ethanolic potassium hydroxide was added. The solution was refluxed for 2hrs with time to shaking. 1ml phenolphthalein was added and titrated with 0.5M hydrochloric acid (HCl). The same process was conducted for blank determination.

$$\text{Saponification value} = \frac{(B-A) \times M \times 56.1}{W}$$

Where;

B = Volume in ml of the standard HCl solution used for the blank test

A = Volume in ml of the standard HCl solution used for sample

M = Molarity of standard HCl (0.5M) or concentration of the standard HCl (0.5M)

56.1 = Equivalent weight of KOH

W = Weight in gram of the test sample (2g)

h. Iodine Value (Number) Determination

0.5g of oil sample was weighed accurately into a 250ml glass stoppered flask and dissolved with 20ml of Carbon tetrachloride. 25ml of Wijs solution was added into the flask containing the sample. The flask was vigorously shaken and allowed to stand in the dark for 1 hour. 20ml of 15% potassium iodide solution was added together with 100ml of deionised water to the flask. The liberated iodide solution was titrated with 1M standard thiosulphate solution, while shaking vigorously, using starch solution (which was yellow in colour) as indicator until the blue colour formed by the addition of the starch solution disappeared. A blank test was carried out simultaneously without the oil under the same conditions.

$$\text{Iodine value} = \frac{(B-A) \times M \times 12.692}{W}$$

Where;

B = Volume in ml of standard thiosulphate used for the blank

A = Volume in ml of standard thiosulphate used for sample

M = Molarity of standard thiosulphate

W = Weight in gram of test sample

12.692 = Atomic weight of iodine (constant)

i. Ester Value Determination

This is a measure of the total glyceride present in the given fat or oil. Acid value is subtracted from the saponification value to get ester value.

Ester value = SV - AV

j. Determination of Specific Gravity

A clean dry density bottle was weighed empty (W_1). The same bottle was filled with tap water and reweighed (W_2). The water was poured out, the bottle dried in the oven at 100^0C for 60min, allowed to cool and then filled with oil and reweighed (W_3). The density of the oil was calculated thus.

$$\text{Specific Gravity} = \frac{W_3 - W_1}{W_2 - W_1}$$

Where;

W_3 = Weight of density bottle + Oil

W_2 = Weight of density bottle + Water

W_1= Weight of density bottle

k. Determination of Density

Density is a measure of the amount of matter contained by a give volume. It is simply mass per unit volume. The oil was weighed in air (mass in gram), then the oil was dropped in a liquid to displace its volume (volume in cm^3).

$$\text{Density} = \frac{\text{mass of oil (g)}}{\text{Volume displaced } (cm^3)}$$

l. Peroxide Value Determination

1.5ml of the oil sample was measured with the conical flask, 10ml of chloroform was added to it and swirl to dissolve oil. 15ml acetic acid and 1.0ml KI solution were added mixed and left for 5minutes in a dark place. 30 ml of distilled water and 1ml of starch indicator solution were added, and titrated with 0.002M sodium thiosulphate ($Na_2S_2O_3$) until a bluish colouration was attained as the end product.

A blank determination (10ml chloroform + 15ml acetic acid + 1.0ml KI + 30 ml H_2O) was carried out. 1ml of starch indicator was added before titrating and titrate drop wise.

$$\text{Peroxide Value} = \frac{V_1\text{-}V_0 \text{ x M x } 10^3}{W}$$

Where,

V_1 = Volume of sodium thiosulphate solution required to titrate the sample

V_0 = Volume of sodium thiosulphate solution required to titrate the blank determination

M = Exact concentration of standard thiosulphate or molarity of sodium thiosulphate

W = Weight/mass of the sample in gram.

m. Determination of Cholesterol Content

0.1ml of the oil sample and standard cholesterol dissolved in chloroform in ratio 1:10 was evaporated to dryness in a water bath at 50°C. Glacial acetic acid (3.0ml) and 3.0ml of colour reagent (a solution of ferric chloride / glacial acetic acid/ sulphuric acid), was added to each sample and the standard, then shaken vigorously to dissolve the oil. Blank contained, 2.0ml of chloroform, 3.0 ml glacial acetic and 3.0ml of colour reagent. After cooling for 30 minutes at room temperature, absorbance of standard and samples were read at 560nm. Cholesterol content was estimated with the formula;

$$\text{Cholesterol mg/ml} = \frac{AB}{AS} \text{ x } \frac{CS}{1}$$

Where,

AB = Absorbance of oil sample

AS = Absorbance of Standard cholesterol

CS = Concentration of Standard cholesterol

n. Elemental Analysis Using Atomic Absorption Spectrophotometer

The sample is thoroughly mixed by shaking and 100ml of it is transferred into a glass beaker of 250ml volume. The sample is aspirated into the oxidizing air – acetylene flame or nitrous oxide acetylene flame. When the aqueous sample is aspirated, the sensitivity for 1% absorption is observed.

o. Fatty Acid Profile Test Using Gas Chromatography

Weigh out about 2g of oil in a small beaker and record the exact weight.

Dissolve the sample in 50ml of chloroform and transfer to a 100 ml volumetric flask and dilute to the mark. Transfer 1ml of unknown sample to a 10ml screwtop culture tube with Teflon liner. Now add exactly 1.00ml of a standard solution of 0.814mg/ml pentadecanoic acid. When you esterify the glycerides in the fat sample, you will also esterify the pentadecanoic acid standard.

(i) The efficiency for esterification of the standard is the same as that of the glycerides.

(ii) the response of the detector to each of the FAMES including the C15 internal standard is the same, then we can quantify the amount of each ester in the fat by comparison of the integrated areas with the known concentration of the standard.

Evaporate most of the chloroform under a stream of nitrogen until ~100 µl of the solution remains. If the solution is dried completely, it will be hard to re-dissolve with the esterification reagent.

Next, add 1 ml of interesterification reagent [25 vol% of a 12% BF3-methanol solution, 20 vol% benzene and 55 vol% methanol]. Flush the tube with nitrogen, seal it, and heat it in a 100°C water bath for 30 minutes. After inter esterification, extract the methyl esters with hexane and H2O so that the final mixture of the reagent, hexane and water, is in a proportion of 1:1:1 (i.e., add 1 ml each of hexane and water to the reaction mixture). Shake the mixture vigorously by hand for 2 min. If a stable emulsion is formed, break it by centrifugation. Transfer about half of the top hexane phase to a small test tube for injection. Be careful to remove only the organic layer. Do not inject directly from the reaction vial because of the risk of injecting water. Water can ruin the Gas Chromatography column.

L. Determination of Vitamins in Rhynchophorus Ferrugineus Larva Oil

a. Determination of Vitamin A

Vitamin A absorbs in the region of the spectrum with a maximum at 325nm. The method is rapid and sensitive but suffers from the disadvantage of low specificity as a result of a number of other substances which absorb in the region of the spectrum as vitamin A, therefore, allowance has to be made for these interfering compounds. One method is to measure the extinction at 325nm before and after irradiating with UV light.

Vitamin A concentration of the sample (mg/ml)

$$= \frac{T_1 - T_2}{ST_1 - ST_2} \times 1 \times \text{dilution factor}$$

b. Determination of Vitamin E

This was determined by the Futter- Mayer colorimetric method with association of Vitamin chemist's (Kirk and Sawyer, 1991). 1g of the sample was mixed with 10ml of ethanoic sulphuric acid and boiled gently under reflux for 30mins. It was transferred to a separating funnel and treated with 3 x 30ml diethyl ether and recovering ether layer each time, the ether extract was transferred to a desiccator and dried under for 30mins and later evaporated to dryness at room temperature. The dried extract dissolved in 10ml of pure ethanol. 1ml of the dissolved extract and equal volume of standard vitamin E were transferred to separate tubes. After continuous addition of 5ml of absolute alcohol and 1ml of concentrated nitric acid solution, the mixtures were allowed to stand for 5mins and the respective absorbance measured in a spectrophotometer at 410nm with blank reagent at zero.

Conc. of Vitamin E in the sample

$$= \frac{\text{Abs of sample x Conc of standard used}}{\text{Weight of Sample Used}}$$

c. Determination of Vitamin C by Titrimetric Method

Prepare 0.001M 2, 6 dichlorophenolindophenol (the dye) and find equivalent point of standard solution of ascorbic acid (10mg/l).

If titre value of dye = xml, then the equivalence of 1ml dye to standard ascorbic acid (using 10ml standard Vitamin C)

$$\text{Equivalence (dye)} = \frac{10\text{mg} \times 10\text{ml}}{}$$

d. Determination of Vitamin B1 (Thiamine) by Colorimetric Method

The method is based on the reaction of thiamine with Reinecke's salt forming a precipitate which can be dissolved in acetone and measured calorimetrically at 525nm. The reagent is a solution of Reinecke salt NH_4 $\{Cr(NH_3)_2(SCN)_4\}.O.66H_2O$. The reference solution is a 0.1% thiamine hydrochloride in 1% HCl. 10 milliliters of a solution of the sample containing between 2mg and 5mg thiamine hydrochloride in p^H of 4.5 acetate buffers are treated with 5mlof Reinnecke salt solution. The precipitate is allowed to form, collected on a sintered glass crucible, washed and dissolved in 10ml of acetone. The solution is made to volume and read at 525nm against a blank and the results are evaluated against a standard curve prepared from the thiamine reference solution in buffer p^H 4.5.

e. Determination of Vitamin D (Calcium Estimate)

To a 50ml of sample inside conical flask was added 4 ml of 8M KOH, swirled at intervals and allowed to stand for 5 mins. Then 3mg each of potassium cyanide and hydroxylamine hydrochloride followed by 0.2g of patton and readers indicator. The resulting solution is titrated with 0.01M EDTA to a blue colour end point.

$$\text{Vitamin D} = \frac{\text{Titre x } 0.4008 \text{ x } 10^6}{\text{Volume of Sample Used}}$$

f. Determination of Boiling Point of Raffia Palm Maggot Oil

30ml of oil sample was measured respectively into a beaker with a measuring cylinder. The beaker containing the oil sample was held with a retort stand over the Bunsen burner and heated to its boiling point. The thermometer was inserted into the beaker, until it maintains a constant temperature. Care was taken not to allow the thermometer touch the beaker. The boiling point was noted.

III. Results

Table I: Percentage Oil Yield of Different Methods of Extraction

Methods of Oil Extraction	*Percentage Oil Yield*
Soxhlet Extraction Method	24.6%
Maceration Method	18.2%
Cold Extraction Method	10.6%

Table II: Results Of Physical Parameters

Physical Parameter	*Value*
Colour	Golden yellow
Odour	It has the aroma of the source
Density	$0.8 g/cm^3$
Specific Gravity	0.8
Viscosity	13.09 pa.s
Appearance	Liquid at room temperature
Moisture Content of maggot	25.82%
Boiling Point	108^oC
Fire Point	193^oC
Flash Point	192.5^oC
Smoke Point	180^oC
Cloud Point	173^oC
Crude Fibre	50.2%
Crude Fat	25%
Crude Protein	11.55%
Ash Content	31.94%
p^H	5.03

Table III: Results of Chemical Parameters

Chemical Parameter	*Value*
Iodine Value	128.1892mg/g
Acid Value	3.366mgKOH/g

Refractive Index	1.0838
Free Fatty Acid Value	1.683mgKOH/g
Saponification Value	257.78mgKOH/g
Ester Value	254.4134mgKOH/g
Peroxide Value	6.93meq/kg
Cholesterol Value	0.2020mg/ml
Test for Unsaturation Using $KMnO_4$	$KMnO_4$ was decolourized
Test for Unsaturation Using Bromine	The colour of bromine was discharged
Glucose	1.04mg/g

Table IV: Result of Fatty Acid Profile Test Using Gas Chromatography

Component	*Name*	*Concentration (ppm)*	*% Concentration*
C20	Arachidic Acid	271.9492	0.3034
C30+	Mellistic Acid	454.2485	0.5069
C17	Magaric Acid	17.2582	0.0192
C18	Methyl Stearate	380.7288	0.4248
C14	Myristate	7.6980	0.0085
C12	Lauric Acid	49515.1952	55.256
C16:2	Palmitoleate	38713.6503	43.202
C16	Palmitic Acid	249.4642	0.2783

Table V: Result of Elemental Analysis Using AAS

Element	*Concentration (ppm)*
Sodium	7.53
Potassium	8.63
Manganese	0.05
Iron	0.23
Mercury	0.00
Copper	0.26
Magnesium	12.61
Nickel	0.18
Lead	0.00
Arsenic	0.00
Calcium	28.32
Chromium	0.00
Cadmium	0.00
Silver	0.00
Cobalt	0.00
Zinc	0.17
Molybdenum	0.00
Aluminium	0.00
Tin	0.00

Table VI: Result of the Test for Vitamins

Vitamin	*Value*
Vitamin A	0.5mg/ml
Vitamin E	0.613mg/l
Vitamin C	0.03mg/10ml
Vitamin D	6.4128mg/l
Vitamin B_1	2.3mg/ml

IV. Discussion

The methods of extraction of the oil sample were shown in Table 1 with soxhlet extraction method giving the highest oil yield.

The physical and chemical properties of *Rhynchophorus ferrugineus* larva oil are presented in Table 2 and 3 respectively. The values obtained are closely related to the standard range approved by Standard Organization of Nigeria (SON). The oil being liquid at room temperature, suggests that the oil is unsaturated.

The physical analysis also revealed that the smoke, flash and fire points of the oil sample was not significantly different. The high values obtained are indicative of the suitability of the oil sample for frying.

The golden yellow colour of the oil is prevalent in high quality edible oils.

$KMnO_4$ being decolorized during test for unsaturation shows that the test sample is unsaturated and also the discharge of the colour of bromine, during bromine test is a clear indication that the oil is unsaturated.

The peroxide value of the oil sample (6.93meq/kg) was in agreement with the maximum Codex Standard peroxide value which is 10meq/kg for vegetable oil deterioration. It shows that *Rhynchophorus ferrugineus* larva oil has significantly high peroxide value which suggests that it has high content of unsaturated fatty acids.

The iodine value which is a measure of the degree of unsaturation in edible oil is 128.1892mg/g, which falls within the range of class of oil known as semidrying oil. It suggests that the oil contains some proportion of unsaturated double bonds.

The acid value of the oil (3.366mgKOH/g) is higher than the acceptable limit of the Codex Standard for vegetable oil which is 0.6mgKOH/g which indicates high free fatty acids and leads

to a tendency of becoming rancid, which resulted probably during processing of the oil. The fatty acids could have reacted with either water, oxygen or some micro organisms might have acted on it.

The high moisture content of raffia palm larva (maggot) (25.82%) will not encourage the storage stability of the maggot because moisture content of food is a measure of index of water activity and is used as a measure of stability and susceptibility to microbial contamination, so the shelf life of the maggot can be improved by sun drying and frying which will bring about dehydration and keep the quality of the maggot meal.

The fat content of the raffia palm maggot is 25% which according to literature is higher than that of termites and egg but lower than that of cow milk (FAO, 1981). The implication of this high fat content is that undefatted larva will be prone to deterioration by peroxidation which may be accompanied by increased browning reaction leading to reduced lysine activity.

The cholesterol value of the oil extract (0.2020mg/ml) falls within the tolerable limit and it suggests that raffia palm maggot oil is healthy for consumption.

The crude fibre content of the raffia larva (50.2%) shows that the maggot is very rich in dietary fibre. According to literature it helps to prevent constipation by increasing the stool weight and decreasing gut transit time. It also slows digestion and absorption of carbohydrates and hence lowers the rise in blood sugar that follows a meal (post prandial and insulin response), this can help people with diabetes improve their blood sugar levels. Fibre provides bulk in the diet without added calories and can give a satiating effect on appetite and also help in weight management.

The high saponification value (257.78mhKOH/g) of the oil extract is an indication that the oil will be most suitable for soap making.

The protein content (11.5%) implies that *Rhynchophorus ferrugineus* larva oil can contribute significantly to the daily human protein requirement.

The Fatty Acid Profile Test by Gas Chromatography summarized in Table 4, revealed a preponderance of saturated and unsaturated fatty acids. The oil contains a large quantity of palmitoleate (C16:2) which is an ester of palmitoleic acid (omega 7 monounsaturated fatty acid) which indicates the presence of 16 carbon chains and two double bonds. So the oil contains mono unsaturated fatty acid as well as saturated fatty acid as a result of the high presence of lauric acid and small quantity of other saturated fatty acids.

The elemental analysis of the raffia palm maggot oil using AAS presented in Table 5 showed that the oil contains Calcium, Magnesium, Potassium and Sodium in large quantity which is indices of nutritive value of food and contains Iron, Copper, Zinc and Manganese in minute quantity. These elements according to literature are known to play beneficial roles to the good health of man and animals. For instance Calcium ions (Ca^{2+}) are necessary for the coagulation of blood, proper functioning of human heart and nervous system and normal contraction of the muscles. Its most important function however is to aid in the formation of bones and teeth (Golden and Golden, 1981). Potassium and Sodium play vital roles in osmotic regulation and maintenance of acid – base balance in the body. Potassium also plays a vital role to the heart rhythm while Sodium is important to fluid balance, acid – base balance, glucose absorption and regulation of muscle and nerve irritability (Kirk and Sawyer, 1991). Magnesium ensures protection from disease such as osteoporosis and hypertension. Copper is essential for proper growth of the body, efficient utilization of iron, proper enzymatic reactions as well as improved health of connective tissues, hair and eyes. Copper is also integral for the prevention of premature ageing and increase in energy production. Iron helps to metabolize proteins and in the production of haemoglobin, enzymes and red blood cells. Zinc plays a vital role in growth, cell division, fertility and immune system. The high concentration values of Magnesium, Calcium, Sodium and Potassium and minute quantities of Manganese, Copper, Zinc and Iron suggests that raffia palm maggot oil can serve as a good food supplement provided it is used within the safe recommended limit.

The preponderance of Vitamin D showed that the oil will serve as a very good dietary supplement for Calcium deficient meals. The oil contains little quantity of vitamin E which is an anti oxidant and according to literature is used as an anti aging portion. Vitamin B_1 level in the oil implies that that it could serve as a good source of Vitamin B_1 in diets. Vitamin C is contained in the oil, therefore it further strengthens its application as a food supplement because according to literature Vitamin C hold the cells together, heal wounds and build bones and teeth. It helps to make collagen, an important tool in wound repair. It is also a strong antioxidant with immune boosting effects.

V. Conclusion

In conclusion, considerable efforts have been made to establish the physical and chemical indices of the oil. The study x- rayed in-depth, the physical and chemical properties of the oil, and came to conclusion that *Rhynchophorus ferrugineus* larva oil satisfies the need of domestic food preparation (nutritional value) industrial raw materials for the manufacture of soaps, cosmetics (industrial value) and can be used as a very good dietary supplement (medicinal value). Considerable efforts have been made herein to establish the physical and chemical indices of the oil.

VI. Recommendation

I recommend the consumption of raffia palm maggot because of its nutritional and medicinal values. From the results of the various analysis carried out on the oil, the oil is strongly recommended as food supplement. It also serves industrial purposes as in the production of soap and cosmetics because of the presence of vitamin E which serves as an anti ageing agent.

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