ANALYSIS OF LARVAL OIL OF *RHYNCOPHORUS PHOENICIS* F

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ABSTRACT

A golden-yellow oil was obtained from the larva of *Rhyncophorus phoenicis* by solvent extraction (yield, 27.2% w/w fresh weight). The oil consisted of neutral lipids, 88.8%; phospholipids, 8.4%, and glycolipids, 2.6%. The fatty acids were analyzed as their methyl esters by gas liquid chromatography. The unsaturated fatty acids were found to predominate over the saturated in the various neutral, phospho - and glycolipid fractions. Major fatty acids (> 5%) in the total lipid are palmitic ($^{C}16:0$) 32.9%, oleic ($^{C}18:2$) 48.3%, and linoleic ($^{C}18:2$) 8.6%. Physical and chemical constants for the oil were determined by standard methods. The larval oil also contained cholesterol, 0.4%, and tocopherol (vitamin E), 0.6%. Vitamins A and D were determined qualitatively. The results indicate that the larval oil might have potential as an edible oil.

INTRODUCTION

Rhyncophorus phoenicis F., a member of the Curculionidae family, is a reddish-brown beetle with two reddish bands on the thorax. It is commonly found on coconut palm, oil palm and raphia palm trees. Other members of the genus include *R. palmarum, R. ferrugineus, R. papau* and *R. Schach.* Of the twelve species of Rhyncophorus known to attack palms, only *R. phoenicis* is found in Africa (Hill, 1969; Mariau 1971). The adult females of *R. phoenicis* lay their eggs in holes and crevices made on the host by man or other insects. Each female is known to lay about 200–500 eggs which are hatched after about three days. The resulting larvae feed voraciously on the trunk of the palm trees damaging the plant tissues and thereby causing extensive economic damage (Mariau, 1968; Singh, 1972).

The larva of *R. phoenicis* is apodous, yellowish-white or creamy in colour, 6–7 cm long, with a reddish-brown capsular head. It is commonly called the "edible worm" in many localities in Nigeria where the larva is considered a delicacy and commonly eaten for its purported nutritional value. The larva may be consumed in the raw state but normally it is boiled or roasted. More frequently it is eaten with tapioca or bread as a standard meal. In traditional medicine practice (especially among the Itsekiris and Urhobos of Bendel State in Nigeria) the larva is commonly prescribed for pregnant women particularly those with a history of habitual abortion, and for infants that are deemed to be malnourished or "habitually weak".

Previous reports on *R. phoenicis* have focused on the economic damage of the larva to various palms (Mariau, 1968 and 1971; Singh, 1972). A study by Fast (1967) reported on the lipid content of another member of the genus, *R. palmarum* but did not include the characteristics of the lipid. Inspite of the acclaimed attributes of the "edible worm", reports on *R. phoenicis* larval oil are still lacking. The objectives of this study were to obtain data on the yield, physical and chemical characteristics of *R. phoenicis* larval oil as well as the fatty acid composition of the total lipids and the various neutral, phospho – and glycolipid components.

EXPERIMENTAL

Preparation of Sample

The larvae of *Rhyncophorus phoenicis* were collected live (in the month of March) from raphia palms at Mosogar in Bendel State of Nigeria. They were identified as such independently by Mr. A. Aisagbonhi of the Entomology Department of the Nigerian Institute for Oil Palm Research (NIFOR) Benin City, and by Dr. (Mrs) B. N. Iloba of the Department of Zoology, University of Benin, Benin City.

After collection, the larvae were rinsed in distilled water to remove superficial matter and then blotted dry before oil extraction.

Extraction of Oil:

28 larvae weighing 150.9g were blended with 200ml Folch reagent consistinf of chloroform-methanol (2:1, v/v) for 2–3 min at room temperature in an Omni-mizer (OCI Instruments, Model 17106). The resulting mixture was filtered with Whatman No. 1 paper in a Buchner funnel under reduced pressure. The residue was re-extracted with another 200ml Folsh reagent, filtered and washed 2 times with 30ml chloroform.

The extracts and washings were combined and transfered quantitatively to a separatory funnel and allowed to stand until the two phases were defined. The upper phase was further extracted 2 times with 30ml chloroform and combined with the lower phase. The combined extract was dried over anhydrous sodium sulphate and filtered. The extract was then concentrated in a rotary evaporator at reduced pressured at 45°C to give a golden-yellow free-flowing oil.

Analytical Procedures:

Oil yield was determined gravimetrically after the extracted oil had been freed of residual organic solvent at room temperature under a flux of nitrogen. The yield was calculated on percentage fresh weight basis and on lipid-free dry weight (lean weight) basis. Lipid-free dry weight being the weight of the air-dried residue after tissue has been exhausted with Folch reagent.

The specific gravity, refractive index, saponification value, unsaponifiable matter and iodine value for the oil were determined by standard methods (IUPAC, 1974).

The oil was fractionated into three lipid classes by two column separations. Polar lipids were separated from neutral lipids by silicic acid column chromatography using a 1.8cm diameter glass column packed with 80 g adsorbent and loaded with 2 g of oil dissolved in a minimal amount of chloroform. Neutral lipids were eluted by chloroform (1500ml) and polar lipids by methanol (1000ml). Each fraction was collected in bulk and concentrated in a rotary evaporator under reduced pressure.

The polar lipid was fractionated into glycolipids and phophohpids based on the method described by Rouser et al (1967). The polar lipid fraction was redissolved in a minimal volume of acetone and transfered quantitatively onto a Florisil column (Florisil 60/100 mesh, Fisher Scientific Co, Fair Lawn, N.J.) consisting of 30g Florisil in a 1.8cm diameter glass column. Glycolipids were eluted by acetone (1000ml) and phospholipids by methanol (400ml). Each fraction was again collected in bulk and concentrated as described earlier.

The purity of each lipid class was monitored by Thin layer Chromatography (TLC) on silica gel G. The developing solvent system for neutral lipids was chloroform and visualization was done separately by charring with 20% Sulphuric acid, and by spraying with phosphomolybdic acid. Chloroform-methanol-water (65:25:4, v/v/v) was used as the developing solvent system for both glycolipids and phospholipids. Molybdenum blue reagent was used for detecting phospholipids, and diphenyl amine solution for glycolipids (Peng, 1974).

A portion of the neutral lipids (820mg was accurately weighed and saponified in 0.5M KOH in 10% aqueous methanol (15ml) by leaving the reaction flask in an oven regulated at 45°C for 4 hr and shaking at intervals of 30 min. After cooling, the unsaponifiable fraction was obtained by extracting 2 times with diethyl ether. The combined ether extract was washed with water and dried over anhydrous sodium sulphate. The ether solvent was removed at room temperature under a steam of nitrogen. The unsaponifiable matter was fractionated by preparative TLC on silica gel Pf 254 + 366. The developing solvent system was benzene-methanol (90:1, v/v). The upper and lower bands were scraped from the plate into separate conical flasks and extracted with chloroform. The extracts were concentrated under nitrogen. The upper band was tentatively identified as tocopherol by CO-TLC with authentic tocopherol and located with Rhodamine

The tocopherol (Vitamin E) content of the oil was further determined by the Furter-Meyer method (Association of Vitamin Chemists, 1966). The lower band on the preparative TLC plate was isolated and identified as cholesterol and located with Liebermann-Burchard reagent.

Qualitative determination of vitamin A (as retinol) in the oil was made on a solution of the unsaponifiable matter using the Carr-Price method (B.P; 1980) based on the reaction between vitamin A and antimony trichloride. Vitamin D (ergocalciferol) was determined qu litatively following the methods described in the B.P. (1980).

Gas Liquid Chromatography (GLC)

Gas chromatographic analyses of fatty acid composition for individual lipid classes were carried out on a Pye Unicam Series 304 gas chromatograph equipped with both flame ionization and electron capture detectors (Pye Unicam Ltd Cambridge, England). Fatty acid methyl esters were prepared by a single step methanolysis with BF_3 -Methanol according to the method of Morrison and Smith (1964). The fatty acid methyl esters were analyzed using a coiled glass column (1.5m x 4mm I.D.) prepacked by Phillips incorporated, with 10% diethyleneglycol adipate (DEGA) coated on Diatomite CAW, 100–120 mesh. The operating conditions were: Column temperature 200°C, injection port temp 250°C, detector temp (FID) 280°C, carrier gas, nitrogen at a flow rate of 40ml/min. The fatty acid methyl ester components were identified provisionally by comparison of the relative retention times ($Rt_{18:0}$) of the peaks with those of reference esters analyzed under the same chromatographic conditions. The fatty acid methyl esters were expressed as peak area percentages of the total peak area from all methyl esters were expressed as peak area percentages of the total peak area from all methyl esters in a given sample. No attempt was made to determine the exact location of the double bonds in the unsaturated fatty acids.

RESULTS AND DISCUSSION

A golden yellow oil with a mild characteristic sweet flavour was obtained from the larva of *Rhyncophorus phoenicis*. Larval oil yield was found to be 27.2% w/w of the fresh weight and 224.8% when expressed as a percentage of lipid-free dry weight (lean weight). Oil yield determined as percentage fresh weight is subject to fluctuation in value due to changes in moisture content of the larva. The procedure for determining oil yield based on dry weight subjects the larval material to high temperature treatment which may lead to profound changes in the nature of the lipid. Expressing oil yield as a percentage of lipid-free dry weight therefore overcomes the inherent shortcomings of the former methods. However, larval oil yield of 27.2% fresh weight is significantly different from the yield of 22.3% wet weight reported by Fast (1967) for *R. palmarum*.

Results of the physical and chemical analyses of *R. phoenicis* larval oil are presented in table 1. These constants are usually useful in determining the identity and usefulness of most oils. *R. phoenicis* larval oil is free-flowing with a solidication value of 8–10°C. This is suggestive of a highly unsaturated oil as was also indicated by the high iodine value of 120– 27. Thus the oil falls into the category of semi-drying oils like soya bean oil, sesame seed oil and corn oil.

The extracted oil was found to contain 88.8 ± 0.1% neutral lipids, 8.4 ± 0.2% phospholipids and 2.6 ± 0.2% plycolipids. (Average of 3 determinations). Glycolipids are typical of photosynthetic tissue. Hence the low glycolipid content was not unexpected since the larva feeds mainly on wood fibres which are virtually devoid of photosynthetic tissue.

Table I: Physical and chemical constants for *R. phoenicis* larval oil.

Assays[a]	Value
Specific gravity	0.908 – 0.912
Refractive index	1.468 – 1.472
Iodine value	120 – 127
Saponification value	187 – 192
Unsaponifiab e matter (%)	1.9

[a]Results from 3 determinations.

The non-saponifiable fraction obtained from the ether extract of the saponified neutral lipids represented 2.1% of the neutral lipid fraction and 1.9% of the total lipids. Most oils and fats of normal purity contain non-saponifiable matter in the range of 2% (Pearson, 1976). The major components of the non-saponifiable matter were tocopherol (vitamin E) 32.4% and cholesterol, 21.6%. These values translate into 0.6% of the entire oil for tocopherol and 0.4% for cholesterol. Vitamins A and D also were qualitatively determined as components of the non-saponifiable fraction.

Gas liquid chromatographic analysis showed that the larval oil contains 37.7% saturated fatty acids which consisted chiefly of palmitic acid. The unsaturated fatty acids contain mainly C_{18} olefinic acids with oleic acid accounting for as much as 48%. This result is in close agreement with that reported by Young (1967) on the fatty acid composition of eighty species of insects. The fatty acid composition of the total, neutral, phospho - and glyco-lipids of the larval oil are shown in table II. The major fatty acids (> 5%) are palmitic, oleic and linoleic. All the lipid fractions contained higher unsaturated fatty acids than saturated as indicated by the unsaturation: saturation ratios (U/S) of 1.65 for total lipids, 1.75 for neutral lipids, 1.06 for phospholipids and 1.04 for glycolipids.

Table I: [b]Percent composition of fatty acids in *R. phoenicis* larval oil[c]

Fatty acid	Total	Neutral	Glycolipids	Phospholipids
Saturated				
$C_{12:0}$(Lauric)	0.1	0.1	–	–
$C_{14:0}$(Myristic)	2.9	2.8	2.9	3.6
$C_{16:0}$(Palmitic)	32.9	31.8	44.8	41.7
$C_{18:0}$(Stearic)	1.8	1.7	1.4	3.2
Unsaturated				
$C_{16:1}$ (Palmitoleic)	0.3	0.3	1.2	–
$C_{18:1}$(Oleic)	48.3	49.9	43.5	33.4
$C_{18:2}$(Linoleic)	8.6	8.3	6.2	12.8
$C_{18:3}$(Linolenic)	2.7	2.8	–	4.0
$C_{20:4}$(Arachidonic)	2.3	2.3	–	1.3
U/S[d]	1.65	1.75	1.04	1.06

[b]Peak area percentage. [d]Ratio of unsaturated to saturated fatty acids
[c]Average of 3 determinations

CONCLUSION

Lipids are important constituents of foods. Their presence, quantity and composition contribute to the taste of the food and also significant to the nutritional and keeping quality. *R. phoenicis* larval oil compares favourably with some well established oils which are useful as salad oils and cooking oils. Furthermore there is a growing demand for unsaturated oils in food since it has been established that populations on diets with high U/S ratio tend to run low blood-cholesterol averages. This means that they are less prone to artherosclerosis and less predisposed to heart attacks. Hence the nutritional significance of highly unsaturated oils in the diet (Swoboda, 1974).

The larva of *R. phoenicis* has been known to constitute part of the staple diet in certain localities in Nigeria. The larval oil may therefore be considered safe in food and animal feed. However the economic feasibility of breeding the larvae for oil production needs to be investigated.

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