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Characterization of Lipids in *Rhynchophorus pheonicis* Larval Oil

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Abstract. *Rhynchophorus pheonicis* larval oil was extracted, and characterized by physical and chemical methods. The lipid content of the larva was 25.30 ± 0.20% (wet weight). The oil was golden-yellow, odourless and fluid at room temperature (26 ± 2 °C). Lipid analysis revealed that the larval oil comprised of 88.40 ± 0.35% neutral lipid, 8.20 ± 0.11% phospholipid and 2.60 ± 0.10% glycolipids. The unsaturated fatty acids accounted for 61.10 % of the total fatty acids whereas, the saturated fatty acids constituted 38.90% of the fatty acids. Further analysis revealed refractive index of 1.30 ± 0.05, specific gravity of 0.89 ± 0.01, solidification value of 12-14 °C, total lipid phosphorus of 31.00 ± 0.25 (µg/g lipid), acid value of 3.50 ± 0.06, iodine value of 123.60 ± 0.24, saponification value of 198.90 ± 0.25, and unsaponifiable matter of 8.60 ± 0.18. These values when compared with that observed in oils which have been considered to be of high quality, and of much use in the pharmaceutical industries, suggested that *R. pheonicis* larval oil may have pharmaceutical potential.

Keywords: *Rhynchophorus pheonicis*, lipids, physical characteristics, chemical characteristics

Introduction

The larva of the beetle *Rhynchophorus pheonicis* is a delicacy in many parts of Nigeria, and other countries in Africa, where it is found. The larva is known by various names by the different ethnic groups who believe it to have high nutritive value as well as certain medicinal properties (Ekpo and Onigbinde, 2005). The mode of preparation for consumption differs among various ethnic groups and they eat it as part of a meal or complete meal with tapioca or bread (Ekpo, 2003).

Other members of the genus include *Rhynchophorus palmarum, R. ferruginous, R. papaunus* and *R. schach. R pheonicis* is diurnal. The female lays its in palm tree holes and crevices made by man or other insects like *Oryctes rhinoceros*. They actively search for cut petioles as their oviposition sites. Each female lays about 200-500 eggs, which hatch after about three days. The larva is yellowish white or creamy in colour. They are legless (apodous), oval in shape with a reddish brown head capsule and are about 4-7 cm long at maturity.

Fat is the chief energy reserve in the insect larvae (Chapman, 1980; Wigglesworth, 1976; Gilmour, 1961). It is usually present in greatest amounts in the mature larvae (Fast, 1970). Although the fat content can reach as high as 40% of wet weight, most insects are studied contained less than 10% of wet weight as lipid. Those species showing a wet weight fat content greater than 10% are primarily phytophagous (Fast, 1970). Qualitatively, the fatty acids found in insects are those known to all higher organisms with the exception of *trans* sorbic acid and tetradecan-1,14-dioic acid. Bowie and Cameron (1965) identified *trans trans* sorbic acid (6:2) as a major constituent of some aphid fats. None was however found in the aphid host plant, while Tamaki (1968) found up to 12% tetradecan-1,14-dioic acid in *Pseudococcus comstocki.* Saturated fatty acids having 15, 17 and 19 carbon atoms are common, but minor constituents of the total larval lipid fatty acids. There is not much data on branched chain cyclic and ethynoid fatty acids in insect larvae. Structural analysis of the common unsaturated fatty acids indicate that these are mainly 16:1, 18:2 and 18:3 fatty acids (Nelson and Sukkestad, 1968; Keith, 1966).

Some species of insects are eaten, as a delicacy in Nigeria, while some are used for traditional medical practice, yet there is very little information on their chemical composition. The use of the larva of *R. pheonicis* is believed to extend beyond the nutritional value. Traditionally, many claim that the larva has medicinal properties (Ekpo and Onigbinde, 2005). The present study was undertaken to provide data on the lipid composition of *R. pheonicis* larval oil as a prerequisite for the subsequent evaluation of its nutritional/ pharmaceutical potential.

Materials and Methods

Sample collection. The larvae of *R. pheonicis* were obtained from Illushi in Edo state. The species were specifically identified in the Entomology Department, Nigerian Institute for Oil Palm Research (NIFOR), Benin city, Nigeria. They were transported together with their wet/moist feed of raphia palm pith in a well-ventilated plastic container and were used within 12 h of collection. Solvents and chemicals used in

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this study were mostly of the analytical reagent grade. The chloroform and methanol were redistilled before being used in this study.

Analyses. Lipid from the larva was extracted by the method described by Bligh and Dyer (1959). The refractive index, specific gravity and solidification values of the larval lipid were determined using the method described in the British Pharmacopoeia (1980). The iodine value of the lipid was determined by the method of Yasuda (1931), while saponification value was determined using the method of Hartman and Antunes (1971). The method described by Pearson (1976) was used to determine the unsaponifiable matter as well as the acid value of the larval oil. Total lipid phosphorus was determined using the modified procedure of Allen (1940). The general fractionation procedure of Rouser *et al.* (1967) was used for the fractionation of the lipid into neutral lipid, glycolipid and phospholipid fractions. Fatty acid methyl esters (FAME) of the larval lipid were prepared using the method of Gunstone (1969). The FAME extracts were co-chromatographed with authentic FAME standards of known structure. The GLC equipment used was a Pye Unicam series 104 GCD equipped with flame ionization detector (FID) and connected to a Hitachi model 056 recorder (Hitachi Ltd, Tokyo, Japan). The stationary phase comprised of 10% polyethylene glycol adipate (PEGA) on acid washed and silanized chromosorb W (100-120 mesh) packed in a 1.5x4 mm (internal diameter) glass column of length 5 feet. The carrier gas (Nitrogen) flowed at 35 ml/min, while the injection, oven and column temperature was 185 °C.

Results and Discussion

Table 1 shows the total lipid extracted from *Rhynchophorus pheonicis* larva. It also shows the various lipid fractions obtained from the larval oil. The neutral lipid fraction is the major fraction in the larval oil.

Table 2 shows the result for the physical and chemical characteristics of *R. pheonicis* larval oil. The larval lipid is a clear odourless, golden-yellow liquid. It has a low solidification temperature and high iodine value indicating a relatively high level of unsaturation of the oil. The acid value is also low.

Table 3 shows the percentage of fatty acid composition of the whole lipid and for the lipid fractions of *R. pheonicis* larva. Palmitic acid is observed to be the most abundant saturated acid, while oleic acid is the most abundant unsaturated fatty acid in the whole lipid and lipid fractions. The larval oil contains more unsaturated fatty acids, which explains the high iodine number and low solidification temperature of the larval oil. The neutral lipid fraction has a fatty acid profile that is similar to that of the whole lipid.

Table 1. Total lipid and lipid fractions in *Rhynchophorus pheonicis* larval oil

Total lipid and lipid fractions	Percentage %*
Total lipid	25.30±0.20 (wet weight)
	66.61±0.35 (dry weight)
Neutral lipid	88.40±0.35
Phospholipid	8.20±0.11
Glycolipid	2.60±0.10

* = results represent the mean ± sem of three estimations.

Table 2. Physical and chemical characteristics of *Rhynchophorus pheonicis* larval oil (± sem)

Parameters	Mean ± SEM*
Physical characteristics	
Refractive index	1.30 ± 0.05
Specific gravity	0.89 ± 0.01
Solidification value (°C)	12-14 °C
Chemical characteristics	
Acid value	3.50 ± 0.06
Iodine value	123.60 ± 0.24
Saponification value	198.90 ± 0.25
Unsaponifiable matter	8.60 ± 0.18
Free cholesterol (mg/100 g lipid)	6.74 ± 0.93
Total cholesterol (mg/100 g lipid)	34.40 ± 23.98
Total phosphorus (mg/g lipid)	31.00 ± 0.25

* = results represent the mean ± sem of three estimations

Many biologists assume that the female of a species always contains more lipids than the males. In insects, this is not so, and among most insects for which data are available; males have more lipids than the females (Fast, 1970). The fat content in insects can reach as high as 41% of wet weight, but three-fourths of known insect species contain less than 10% of wet weight as lipid. Most species showing a wet weight fat content greater than 10% are primarily phytophagous. This group also includes parasitic and saprophytic species. Mean lipid content on a dry weight basis is about 30% for the larva and 20% for adult (Fast, 1970). The lipid content of *R. pheonicis* (Table 1) is in agreement with this statement. Fast (1966), reported the lipid content for *R. palmarum* as 22.3% of fresh weight of the larva. Lipid value reported for some insects are 3.1% and 4.0% respectively for the larva and adult beetle of *Lachnosterna* species (Davis, 1918), 2.1% for the Japanese beetle *Popillia japonica* (Fleming, 1968), 15.5% for the pupae of housefly *Musca domestica* (Calvert *et al.*, 1969), 7.54% for adult honey bees *Apis mellifera* L.

Table 3. Percentage composition of fatty acids and lipid fractions in *Rhynchophorus pheonicis* larval oil

Fatty acids	Total lipid* %	Neutral lipid* %	Phospholipid* %	Glycolipid* %
Lauric acid (C 12:0)	0.20 ± 0.03	0.20 ± 0.03	--	--
Myristic acid (C 14:0)	3.20 ± 0.12	3.20 ± 0.15	5.2 ± 0.21	--
Palmitic acid (C 16:0)	32.40 ± 0.58	31.70 ± 0.82	35.30 ± 0.57	43.50 ± 0.59
Palmitoleic acid (C 16:1)	3.30 ± 0.20	3.30 ± 1.10	5.50 ± 0.36	6.90 ± 0.21
Stearic acid (C 18:0)	3.10 ± 0.13	2.80 ± 0.21	6.10 ± 0.73	--
Oleic acid (C 18:1)	40.10 ± 0.72	40.00 ± 2.30	42.80 ± 0.70	49.60 ± 1.55
Linoleic acid (C 18:2)	13.00 ± 0.20	13.50 ± 0.06	5.10 ± 0.12	--
Linolenic acid (C 18:3)	3.50 ± 0.10	3.70 ± 0.26	--	--
Arachidonic acid (C 20:4)	1.20 ± 0.04	1.60 ± 0.10	--	--

* = results represent the mean ± sem of three estimations

Table 4. Percentage of saturated/ unsaturated fatty acids in different lipid fractions of *Rhynchophorus pheonicis* larval oil

Parameters	Total lipid %	Neutral lipid %	Phospholipid %	Glycolipid %
TUFA	61.10	62.10	53.40	56.50
TSFA	38.90	37.90	46.60	43.50
MUFA	43.40	43.30	48.30	56.50
PUFA	17.70	18.80	5.10	--

TUFA = total unsaturated fatty acid; TSFA = total saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid

(Ryan *et al.*,1983), 23.22% (dry matter basis) for *Anaphe venata* (Ashiru, 1988) and 14.87% (fresh weight) for the larva of *Oryctes rhinoceros* (Ekpo, 2003). Comparatively, the lipid value of *R. pheonicis* larva is higher than that found in most insects for which data are available (Fast, 1970), and when compared to lipids derived from conventional foods of animal origin (Pyke, 1979), is found to be higher. Malnutrition in developing countries is as much, or more, a problem of calories deficiency as protein deficiency (DeFoliart, 1992); the consumption of these insects could go a long way in taking care of the calorie needs in such communities. Table 2, shows the physical and chemical characteristics of *R. pheonicis* larval oil, which is a clear golden-yellow and odourless liquid, with a low solidification temperature when compared to values reported for some other oils (Pearson, 1976).

The iodine value of 123.60 ± 0.24 is quite high. High iodine value is a common feature of most insect larval lipids as reported for silkworm oil 117, lepidopterous larva between 112 -159 and 108.6-118 in *Phytophagous chrysomelids* (Wigglesworth, 1976). The specific gravity and refractive index of the larval oil (Table 2) is lower than those for arachis oil, linseed oil and olive oil (Pearson, 1976). This implies that the oil from this insect is lighter than those oils that have been considered to be of high quality and as such find much use in the pharmaceutical industries. In addition, it has more unsaturated fatty acid than those oils, which suggests that it would be more fluid at room temperature and less viscous in low temperatures. The lower acid value is also an indication of its lower susceptibility to rancidity. These observed characteristics suggest that R. *phoenicis* larval oil may be useful as a vehicle for oily infections in the pharmaceutical industries. However, there is need for confirmation by trials in animal models. The unsaponifiable fraction in the oil is 8.60 ± 0.18 which is quite high when compared to 1.5-1.6% in *Bombyx mori* (Bergmann, 1937) and 1.56% in the meal worm *(Tenebrio col)* (Becker, 1934). One-third of these unsaponifiable fractions is sterol of which 85% is cholesterol (Bergmann, 1937). Sterols (especially cholesterol) are essential in insects for normal growth, metamorphosis and reproduction (Thompson *et al.*, 1973). The free and total cholesterol value for the larval oil is 6.74 and 34.4 (mg/100 g lipid), respectively. These values are low compared to values reported for some conventional foods of animal origin. Ritter (1990), attributes the levels of sterol in insects to species difference and diet. Fractionation of the larval oil (Table 1) reveals the neutral lipid fraction as the major lipid fraction followed by the phospholipid fraction. This result agrees with observed results for other insect oils (Fast, 1970). The neutral lipid fraction comprises

mainly of triacylglycerols, an indicator of the high caloric value of the larval oil. Gas-liquid chromatographic analysis confirms the high level of unsaturation of the larval oil. Palmitic and oleic acids are the major fatty acids in the oil. The level of unsaturation in *Rhynchophorous pheonicis* larval oil (Table 4) is higher than for palm oil and coconut oil, which are common household oils. Insect fatty acids are similar to those of poultry and fish in their degree of unsaturation, with some groups being higher in linoleic, linolenic acids, which are the essential fatty acids (DeFoliart, 1991). Niemierko (1947), observed that linoleic acid is usually formed in large amounts in larvae before pupation which suggests hormonal regulation of fatty acid synthesis. The presence of the essential fatty acids such as linoleic, linolenic and arachidonic acid further points to the nutritional value of the larval oil as edible oil. One implication of the high fat content in the larva is that it may increase susceptibility of the undefatted larva to storage deterioration via lipid oxidation (Greene and Cumuze, 1982). This may then be accompanied by increased browning reactions concurrent with reduced lysine availability (Pokorny, 1981). Another implication of the high fat content is that defatting the larva will markedly increase the relative proportions of the other nutrients encompassed in the proximate composition.

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