

Full Length Research Paper

Pharmaceutical potentials of the oils of some popular insects consumed in southern Nigeria

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Accepted 13 March, 2011

Macrotermes bellicosus (MB), *Imbrasia belina* larva (IBL), *Oryctes rhinoceros* larva (OR) and *Rhynchophorus phoenicis* (RP) larva oils were extracted, and the oils were physically and chemically characterized. The lipid content recorded for the insects were $31.46 \pm 0.57\%$, $15.16 \pm 0.18\%$, $14.87 \pm 0.33\%$ and $23.30 \pm 0.33\%$ (wet weight) for MB, IBL, OR and RP respectively. RP and OR insect oils were golden yellow, odourless and fluid at room temperature ($26 \pm 2^\circ\text{C}$), while that extracted from IBL and MB were of a lighter yellow colour. The insect lipids all gave a low solidification temperature and high iodine number indicating a relatively high level of unsaturation of the insect/larval oils. Their saponification values were high suggesting the presence of a fair amount of fatty acids but their acid values were low pointing to the fact that these fatty acids were not free but esterified acids. The cholesterol values were also low but highest in MB with a value of 41.8 ± 0.15 mg/100 g lipid. For all the insects, the neutral lipid fraction was the major fraction in the insect oils. RP had the highest neutral lipid fraction of 88.40 while MB had the least value of 69.87. At the same time MB had the highest phospholipids and glycolipid fractions with values of 19.14 and 10.81 respectively while RP had the least phospholipids and glycolipid fractions with values of 8.20 and 2.60 respectively. For IBL, RP and OR (which are insect larvae) the major fatty acids in the oils were palmitic and oleic acids while for MB (mature insect) the major fatty acids were palmitic and linoleic acids. The insect/larval oils contained more unsaturated fatty acids which explained the high iodine number, low solidification values and the liquid nature of the oils at room temperature. OR recorded the highest level of unsaturation of 65.61 while MB had the least level of unsaturation of 50.02%. Further analysis revealed a refractive index ranging from 1.1 ± 0.01 to 1.3 ± 0.05 , specific gravity of 0.84 ± 0.02 to 0.90 ± 0.01 , solidification value of $10 - 14^\circ\text{C}$, total lipid phosphorus ranging from 31.0 ± 0.25 to 47.18 ± 0.03 $\mu\text{g/gm}$ lipid, acid value of 3.12 ± 0.55 to 3.6 ± 0.06 , iodine value of 108 ± 0.15 to 140 ± 0.51 , saponification value of 187.17 ± 0.55 to 198.9 ± 0.25 and unsaponifiable matter of 8.11 ± 0.02 to 12.04 ± 0.11 . These values when compared with that observed in oils which have been considered to be of high quality and of much use in pharmaceutical industries suggest that these insect oils may have pharmaceutical potential.

Key words: *Macrotermes bellicosus*, *Imbrasia belina*, *Oryctes rhinoceros*, *Rhynchophorus phoenicis*, pharmaceutical potential.

INTRODUCTION

The practice of eating insects or their larva (entomophagy) is not new. Archaeological evidence tells us that entomophagy has been practiced since mankind first made an appearance on this planet. Insects are very abundant and contain many nutrients that are essential to

humans. For example, they are known to have the same amino acid requirements as man (Gilmour, 1961), and so they actively accumulate these amino acids thus being a readily available source of these useful nutrients. It is well known that insects are an attractive and important natural source of food for many kinds of vertebrate animals including birds, lizards, snakes, amphibians, fish and other mammals (McHargue, 1917; Frost, 1942). McHargue cited a U.S. biological survey investigation of

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the stomach contents of 14 species of wild birds revealing that approximately 50% of their annual food consumption consisted of insects. Insects have played an important part in the history of human nutrition in Africa, Asia and Latin America (Bodenheimer, 1951). They were an equally important resource for the Indians of western North-America, who, like other indigenous groups expended much organization and effort in harvesting them (Sutton, 1988).

Fat is the chief form in which energy is stored in insect larvae (Gilmour, 1961; Wigglesworth, 1976; Chapman, 1980). It is usually present in greatest amounts in the mature larvae before metamorphosis (Fast, 1970). Many biologists assume that the female of a species always contains more lipids than the male. 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% ww, but $\frac{3}{4}$ of known insect species contains less than 10% ww as lipid. Most species showing a wet weight (ww) fat content of greater than 10% are primarily phytophagous. This group also includes parasitic and saprophytic species. Mean lipid content on a dry weight bases is about 30% for the larva and 20% for adult (Fast, 1970). Qualitatively, the fatty acids found in insects are those known to all higher organisms with two exceptions; Bowie and Cameron (1965) identified transsorbic 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 lipid fatty acids. There is not much data on branched chain cyclic and ethynoid fatty acids in insects. Structural analysis of common unsaturated fatty acids indicated that these are mainly palmitoleic, oleic, linoleic and linolenic acids (Keith, 1966; Nelson and Sukkestad, 1968; Calvert et al., 1969; Fast, 1970; Thompson, 1973; Stanley-Samuelson et al., 1988; DeFoliart, 1991).

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. In order to be able to accurately evaluate the nutritional and pharmaceutical potential of these "delicacies", it is necessary to carry out detailed analyses of the insects concerned in order to determine their constituents. The present study was undertaken to provide data on the lipid composition of four popular insects consumed in southern Nigeria as a pre-requisite for the subsequent evaluation of the pharmaceutical potentials of these insect oils.

MATERIALS AND METHODS

Rhynchophorus phoenicis (F) and *Oryctes rhinoceros* larvae were obtained live from Ilushi (on the bank of River Niger) in Edo state, Nigeria. *Imbrasia belina* larvae were obtained from Ogbomoso in Oyo state, Nigeria while *Macrotermes bellicosus* was obtained dur-

ing their nuptial flight at Ekpoma, Edo state, Nigeria. The various species were identified in the Entomology Department, Nigeria Institute for Oil Palm Research (NIFOR), Benin City, Nigeria. All live insects/insect larvae were used within 12 h of collection. *M. bellicosus* (termites) were dewinged before being used.

Solvents and chemicals used in this study were mostly of the analytical reagent grade and were obtained from E. Merck (Darmstadt, West Germany), May and Baker (Dagenham, Essex, England), Sigma Chemicals Company (St. Louis, Missouri, U.S.A.). The chloroform and methanol were redistilled before being used in this study.

The lipid from the Insect/ larva was extracted with chloroform-methanol (1: 2, v/v) mixture as described by Bligh and Dyer (1959).

The refractive index and specific gravity of the insect lipids and determination of the solidification value of lipid extracts were determined using the method described by the British Pharmacopia (1980). The iodine value of the lipid extract was determined by the method of Yasuda (1931), as described by Kates (1972). The saponification value of the lipid extract was determined by the method of Hartman and Antunes (1971) as described by Pearson (1976). Determination of unsaponifiable matter was carried out using the method of Pearson (1976). The acid value was determined as described by Pearson (1976). The modified procedure of Allen (1940) as described by Kates (1972) was used for the determination of total lipid phosphorus. The method of Courchaine et al (1959) was used for the determination of total cholesterol. Free and unesterified cholesterol was determined by the method described by Zlatkis et al. (1963). The general fractionation procedure by Rouser et al. (1967) as described by Kates (1972) was used for the fractionation of the insect/larval lipids into neutral lipid, phospholipids and glycolipid fractions. Fatty acid methyl esters (FAME) of the whole lipid (unfractionated) and of the various lipid fractions were prepared by the method described by Gunstone (1969) using methanol/benzene/concentrated H₂SO₄ (20:10:1, v/v/v) mixture as the methylating solvent. The GLC equipment used was a PYE UNICAM series 104 GCD equipped with flame ionization detector (F. I. D) 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 1.5 x 4 mm (internal diameter) glass column of length 5 ft. The carrier gas (nitrogen) flowed at 35 ml/min while injection, oven and column temperature was 185°C. The FAME extracts were co-chromatographed with authentic FAME standards (sigma chemicals) of known structures. Protein nitrogen was estimated according to the Kjeldahl William colorimetric method, (William, 1964). The moisture content of the live larva was determined using the method of the Association of Official Analytical Chemists (AOAC, 1975) as described by Pearson (1976). The method described by Pearson (1976) was used to determine the ash content of the insect/ larvae. Carbohydrate content of the insect/larval samples was determined by difference.

RESULTS

Table 1 shows the proximate composition of IBL, RP, OR and MB. RP had the highest moisture value while MB had the least moisture content. Lipid values revealed that RP had the highest lipid value of 25.30% and when dehydrated the lipid value rose to 66.61%. IBL had the least lipid value of 15.16% (wet weight) but after dehydration the lipid value rose to 23.38%. The protein values observed in IBL were higher than for all the other insects studied with a value of 35.18%. This is closely followed by MB with 33.41%. Dehydration and eventually

Table 1. Proximate composition (% wet weight) of some insects consumed in southern Nigeria.

Insect	Moisture	Lipid	Protein	Carbohydrate	Ash
IBL	34.36 ± 0.25	15.16 ± 0.18	35.18 ± 0.10	7.12 ± 0.55	7.38 ± 0.11
		(23.38 ± 0.24)	(54.26 ± 0.16)	(10.98 ± 0.46)	(11.38 ± 0.08)
RP	61.85 ± 0.18	25.30 ± 0.20	70.81 ± 0.27 ⁺	14.33 ± 0.20 ⁺	14.86 ± 0.15 ⁺
		(66.61 ± 0.35)	(22.06 ± 0.26)	(5.53 ± 0.17)	(5.79 ± 0.13)
OR	60.56 ± 0.41	14.87 ± 0.33	8.38 ± 0.31	2.10 ± 0.10	2.20 ± 0.08
		(38.12 ± 1.06)	(66.09 ± 0.28 ⁺)	(17.16 ± 0.41)	(17.35 ± 0.08 ⁺)
MB	12.60 ± 0.26	31.46 ± 0.57	11.76 ± 0.90	6.87 ± 0.24	5.51 ± 0.19
		(36.12 ± 0.28)	(30.15 ± 1.10)	(14.25 ± 0.19)	(11.26 ± 0.11)

Results represent the mean ± SEM of three estimations; Values in brackets are % dry weight; ⁺ values are % lean weight of the larvae; OR = *O. rhinoceros*, IBL = *I. belina*; MB = *M. belicosus*, RP = *R. phoenicis*.

Table 2. Physical characteristics of lipids in some insects consumed in southern Nigeria.

Insect	IBL	RP	OR	MB
Specific gravity	0.84 ± 0.02	0.89 ± 0.01	0.88 ± 0.01	0.90 ± 0.01
Solidification value	12 - 14°C	12 - 14°C	10 - 14°C	10 - 14°C
Refractive index	1.2 ± 0.01	1.3 ± 0.05	1.1 ± 0.01	1.2 ± 0.01

Results represent the mean ± SEM of three estimations; OR = *O. rhinoceros*, IBL = *I. belina*; MB = *M. belicosus*, RP = *R. phoenicis*.

Table 3. Chemical characteristics and cholesterol content of some insects consumed in southern Nigeria.

Insect	IBL	RP	OR	MB
Acid value	3.3 ± 0.13	3.5 ± 0.06	3.12 ± 0.55	3.6 ± 0.06
Iodine value	131 ± 0.25	123.6 ± 0.24	140 ± 0.51	108 ± 0.15
Saponification value	187.17 ± 0.55	198.9 ± 0.25	190 ± 0.21	193.4 ± 0.31
Unsaponifiable matter	8.11 ± 0.02	8.6 ± 0.18	8.91 ± 0.10	12.04 ± 0.11
Free cholesterol (mg/100g lipid)	5.83 ± 0.41	6.74 ± 0.93	6.81 ± 0.36	8.73 ± 1.01
Total cholesterol (mg/100g lipid)	31.3 ± 0.80	34.4 ± 23.98	36.6 ± 0.17	41.8 ± 0.15
Total phosphorus (mg/g lipid)	36.42 ± 0.50	31.0 ± 0.25	34.8 ± 0.17	47.18 ± 0.03

Results represent the mean ± SEM of three estimations; OR = *O. rhinoceros*, IBL = *I. belina*; MB = *M. belicosus*, RP = *R. phoenicis*.

defatting is seen to increase relative concentration or proportion of the other nutrients encompassed in the proximate composition. The carbohydrate and ash values were highest in MB while RP larva had the least values.

The physical and chemical characteristics of the insect/larval oils are shown in Tables 2 and 3. RP and OR larval oils were observed to be clear, odourless, golden-yellow liquids, while that extracted from IBL and MB was of a lighter yellow colour. The insect lipids all gave a low solidification temperature and high iodine number indicating a relatively high level of unsaturation of

the insect/larval oils. Their saponification values were high suggesting the presence of a fair amount of fatty acids but their acid values were low pointing to the fact that these fatty acids were not free but esterified acids. The cholesterol values were also low but highest in MB.

Table 4 shows the lipid fractions of the insect/ larvae. For all the insects, the neutral lipid fraction was the major fraction in the insect oils. RP had the highest neutral lipid fraction of 88.40 while MB had the least value of 69.87. At the same time MB had the highest phospholipids and glycolipid fractions with values of 19.14 and 10.81 res-

Table 4. Lipid fractions in some insects consumed in southern Nigeria.

Insect	Neutral lipid	Phospholipid	Glycolipid
IBL	85.10 ± 0.31	9.87 ± 0.35	4.16 ± 0.21
RP	88.40 ± 0.35	8.20 ± 0.11	2.6 ± 0.10
OR	83.60 ± 0.20	9.48 ± 1.03	6.81 ± 0.05
MB	69.87 ± 0.73	19.14 ± 0.06	10.81 ± 0.40

Results presents the mean ± SEM of three estimations; OR = *O. rhinoceros*, IBL = *I. belina*; MB = *M. belicosus*, RP = *R. phoenicis*.

Table 5. Percentage composition of fatty acids in some insects consumed in southern Nigeria.

Fatty acids	IBL	RP	OR	MB
C12 : 0	0.12 ± 0.03	0.20 ± 0.03	0.09 ± 0.03	1.50 ± 0.28
C14 : 0	1.15 ± 0.45	3.20 ± 0.12	3.50 ± 0.10	2.17 ± 0.06
C16 : 0	31.90 ± 0.28	32.40 ± 0.58	28.70 ± 0.32	42.45 ± 0.20
C16 : 1	1.80 ± 0.17	3.30 ± 0.20	4.41 ± 0.18	2.10 ± 0.02
C18 : 0	4.71 ± 0.21	3.10 ± 0.13	2.10 ± 0.03	2.86 ± 0.10
C18 : 1	34.20 ± 0.11	40.10 ± 0.72	41.50 ± 2.91	15.84 ± 0.40
C18 : 2	6.02 ± 0.73	13.00 ± 0.20	14.10 ± 0.31	24.24 ± 1.08
C18 : 3	19.60 ± 0.06	3.50 ± 0.10	1.50 ± 0.05	3.90 ± 0.60
C20 : 4	0.50 ± 0.41	1.20 ± 0.04	4.10 ± 0.61	4.94 ± 0.15

Results represents the mean ± SEM of three estimations; C12:0 = Lauric acid, C14:0 = Myristic acid, C16:0 = Palmitic acid, C16:1 = Palmitoleic acid, C18:0 = Stearic acid, C18:1 = Oleic acid, C18:2 = linoleic acid, C18:3 = Linolenic acid and C20:4 = Arachidonic acid. OR = *O. rhinoceros* IBL=*I. belina*; MB = *M. belicosus*, RP = *R. phoenicis*.

Table 6. Degree of saturation of lipids in some insects consumed in southern Nigeria.

Parameter	IBL	RP	OR	MB
TUFA	62.12	61.10	65.61	51.02
TSFA	37.88	38.90	34.39	48.98
MUFA	36.00	43.40	45.91	17.94
PUFA	26.12	17.70	19.70	33.08

TUFA = Total unsaturated fatty acids; TSFA = Total saturated fatty acids MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; OR= *O. rhinoceros* , IBL= *I. belina*, MB= *M. belicosus*, RP= *R. phoenicis*.

glycolipid fractions with values of 8.20 and 2.60 respectively. These results are similar to values reported for other insects for which data are available (Fast, 1970).

The fatty acid composition of the insect/larval lipids is shown in Table 5. For IBL, RP and OR (which were insect larvae) the major fatty acids in the oils were palmitic and oleic acids while for MB (full grown insect) the major fatty acids were palmitic and linoleic acids.

Table 6 shows the degree of saturation/unsaturation of the insect lipids. The insect/larval oils contained more unsaturated fatty acids which explains the high iodine number, low solidification values and liquid nature of the oils at room temperature. OR recorded the highest level

of unsaturation of 65.61 while MB had the least level unsaturation of 50.02%.

Table 7 compares the insect and insect larvae (OR, MB, IBL and RP) fatty acids composition with those for some other insect larvae that have been studied. Results indicate that palmitic acid and oleic acids are the major fatty acids in these insect larvae. These results are in agreement with that observed in the present study.

Table 8 compares the physical and chemical characteristics of OR, MB, IBL and RP larval oil with that for some pharmaceutically important oils such as olive, *Arachis* and linseed oils. Results observed indicate that these insect oils may be pharmaceutically important

Table 7. Comparison of insect fatty acids with those from the larva of other insects (% fatty acid composition).

INSECT	Fatty acid (% composition)									
	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4
<i>Altica ambiens alni</i>	-	1.4	-	22.6	1.6	7.6	23.3	7.6	36.1	-
<i>Euryynassa</i> spp.	-	-	-	29.0	4.0	-	65.0	-	-	-
<i>Chrysomela crotchi</i>	4.2	4.0	-	24.2	1.5	4.7	31.1	5.5	29.4	-
<i>Fornex</i> sp	-	5.1	-	30.7	8.7	1.4	46.5	7.3	-	-
<i>Amphimall mejalis</i>	-	2.3	-	25.4	14.2	4.2	34.2	12.5	3.1	-
<i>Tenebrio molitor</i>	-	1.5	-	23.6	4.5	1.4	44.7	24.1	1.5	-
RP±	0.2	3.2	-	35.4	5.3	3.1	45.1	4.0	2.5	1.2
MB±	1.50	2.17	-	42.5	2.10	2.86	15.8	24.2	3.90	4.94
IBL±	0.12	1.15	-	31.9	1.80	4.71	34.2	6.02	19.60	0.50
OR±	0.09	3.50	-	28.7	4.41	2.10	41.5	14.1	1.50	4.10

± Excluding these values. C12:0 = Lauric acid, C14:0 = Myristic acid, C14:1 = Myristoleic acid, C16:0 = Palmitic acid, C16:1 = Palmitoleic acid, C18:0 = Stearic acid, C18:1 = Oleic acid, C18:2 = linoleic acid, C18:3 = Linolenic acid and C20:4 = Arachidonic acid. OR = *O. rhinoceros*, IBL = *I. belina*, MB = *M. belicosus*, RP = *R. phoenicis*.

Table 8. Comparison of adult and larval insect oil with some pharmaceutically important oils.

Property	Olive oil	Arachis oil	Linseed oil	RP oil ±	MB oil ±	IBL oil ±	OR oil ±
Specific gravity	0.910 -0.916	0.914 -0.917	0.931 -0.936	0.890 - 0.896	0.890 - 0.910	0.820 -0.860	0.870 -0.890
Refractive index	1.468 -1.471	1.460 -1.465	1.479 -1.484	1.280 -1.300	1.190 - 1.210	1.190 -1.210	1.090-1.110
Iodine value	75 -94	80 -105	175	123 -125	107-109	130 -133	139 -143
Saponification value	184 -196	187 -196	188.00	198 -199.30	193.09 -194.00	186.62 -188.00	189.79-191.00
Un-saponifiable matter	15	10	1.5	8 -9	11 -12	8 - 9	8 -9
Solidification value (°c)	-	-	15	12 -14	10 -14	12 -14	10 -14
Acid value	17	4	-	3.30 -3.60	3.54 -3.66	3.17 -3.43	3.06 -3.18

±Excluded; OR= *O. rhinoceros*, IBL=*I. belina*, MB= *M. belicosus*, RP= *R. phoenicis*.

based on the observed characteristics.

DISCUSSION

In Africa, entomophagy is a traditional and culturally acceptable way by which low income persons supplement the meager protein content of their high carbohydrate diets. The interest in the use of insects as food has been expressed in several reports (Umoh et al., 1980; Dreyer and Wehmeyer, 1982; Ukhun and Osasona, 1985; Ashiru, 1988; Onigbinde and Adamolekun, 1998; Ekpo, 1989, 2003; Ekpo and Onigbinde, 2004, 2005, 2007). These insects are usually eaten as part of a meal or complete meal.

Fat is the chief form in which energy is stored in insect larvae (Chapman, 1980). It is usually present in greatest amount in the mature insect larva before metamorphosis (Fast, 1970). The lipid content recorded for the insects were 31.46 ± 0.57 , 15.16 ± 0.18 , 14.87 ± 0.33 and 23.30

$\pm 0.33\%$ (wet weight) for MB, IBL, OR and RP respectively, while on a dry weight or moisture free basis, their lipid contents are 36.12 ± 0.28 , 23.38 ± 0.24 , 38.12 ± 1.06 and $66.61 \pm 0.35\%$ for MB, IBL, OR and RP respectively. These lipid values are quite high when compared with values reported for some other insects and insect larvae. For example, 22% (dry weight) was reported for *Calliphora*, 41 - 45% (dry weight) in *Ophyra cadaverina*, 43.6% (dry weight) in *Galleria* and 30% (dry weight) in the mature larva of *Lucilia* (Wigglesworth, 1976).

The insect oils were observed to be a clear golden yellow or light yellow and odourless liquid with a low solidification value (10 - 14°C) and high iodine value (which is an indication of the degree of unsaturation of the insect oil). The insect oils under consideration were also observed to contain a high amount of unsaturated fatty acids (51.02, 62.12, 65.61 and 61.10 for MB, IBL, OR and RP respectively) which explains the liquid state of the

oils at room temperature ($26 \pm 2^\circ\text{C}$).

High iodine value is a common feature of most insect larval lipids as reported for silkworm oil which has a value of 117, lepidopterous larvae between 112 - 119 and 108.6 - 118 in phytophagous *Chrysomelids* (Wigglesworth, 1976). The level of unsaturation in these insects and larval oils is higher than that for palm oil, coconut oil, beef fat, lard and mutton fat but slightly lower than those for herring, salmon and mackerel (Pyke, 1979). Nutritionally, a high level of saturated fatty acids in food might be undesirable because of the association of saturated fatty acids with incidences of arteriosclerotic disorders (Reiser 1973; Rahman et al., 1995). The presence of essential fatty acids such as linoleic linolenic, and arachidonic acids further points to the nutritional value of these insects and larval oils.

The specific gravity and refractive index for MB, IBL, OR and RP oils are lower than those for *Arachis* oil, linseed oil, and olive oil (Table 8). This means that the insect and larval oils are lighter than these oils which have been considered to be of high quality and of much use in pharmaceutical industries. In addition, these insect oils are more unsaturated than these oils, which suggest that they will be more fluid at room temperature and less viscous in low temperatures. These insect oils are also observed to have a lower acid value when compared with other oils presently in use for oily injections as well as been less susceptible to rancidity. The peroxide values were however not determined.

These listed characteristics and observations suggest that the insects and insect larval oils may be useful as vehicles for oily injections in pharmaceutical industries. However, this would need to be confirmed by further experimentation and trials in animal models.

REFERENCES

- Allen RJ L (1940). The estimation of phosphorus. *Biochem. J.* 34: 858-865.
- Ashiru MO (1988). The food value of the larva of *Anaphe Venata* *Butler. Lepidoptera: Notodontidae*. *Ecol. Food Nutr.* 22:313-320.
- Association of Official Analytical chemists (AOAC) Official methods of Analysis. 12th edition, Washington D.C. (1975).
- Bligh EG, Dyer WJ (1959). A Rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911-917.
- Bodenheimer FS. (1951) *Insects as Human Food*. The Hague: W. Junk (Ed) p. 352.
- Bowie JH, Cameron DW (1965). Colouring matters of the Aphididae (XXV). A Comparison of Aphid constituents with those of their host plants. A glyceride of sorbic acids. *J.Org. Chem.* 5651-5657.
- British Pharmacopia (1980). Volume 11. Her majesty's stationary office (publishers). London.
- Calvert CC, Martin RD, Morgan NO (1969). House fly pupae as food for poultry. *J. Econ. Entomol.* 62: 938-939.
- Chapman RF (1980) *The insects: structure and function*. The English language book society. Stoughton and Hodder. Printed in Great Britain for Hodder and Stoughton Educational. pp. 83-106.
- Courchaine AJ, Miller WH, Stein JRDB (1959) Determination of free and unesterified cholesterol. *Clin. Chem.* 5: 609.
- DeFoliart GR (1991) Insect fatty acids: Similar to those of poultry and fish in their degree of unsaturation, but higher in the polyunsaturates. *Food Insects Newslet.* 4(1): 2-5.
- Dreyer JJ, Wehmeyer AS (1982). On the nutritive value of Mopani worms. *South African J. Sci.* 78: 33-35.
- Ekpo KE (1989). Biochemical Analysis of "Edible Worm" the larva of *Rhynchophorus phoenicis* (F) M.Sc thesis submitted to the Biochemistry Department, University of Benin, Benin-city. Edo state, Nigeria.
- Ekpo KE (2003). Biochemical Investigation of the Nutritional Value and Toxicological Safety of Entomophagy in Southern Nigeria. Ph.D Thesis. Ambrose Alli University, Ekpoma. Edo State, Nigeria.
- Ekpo KE, Onigbinde AO (2004). Pharmaceutical potentials of *Rhynchophorus phoenicis* larval oil. *Nig. Annals Natur. Sci.* 5: 28-36.
- Ekpo KE, Onigbinde AO (2005). Nutritional potentials of *Oryctes rhinoceros* larva. *Nig. J. Nutr. Sci.* 26: 54-59.
- Ekpo KE, Onigbinde AO (2007). Characterization of Lipids in Winged Reproductives of the Termite *Macrotermis bellicosus*. *Pak. J. Nutr.* 6(3): 247-251.
- Fast PG (1970). Insect lipids In: progress in the chemistry of fats and other lipids. 11(2): 181-242.
- Frost SW (1942). *General Entomology*. New York, McGraw Hill. pp. 62-63.
- Gilmour D (1961). *Biochemistry of insects*. Academic Press, New York and London. pp. 84-87.
- Gunstone FD (1969). In: An introduction to the Chemistry and Biochemistry of fatty acids and their glycerides. Chapman and Hall Ltd., U.K. pp. 65-67.
- Hartman L, Antunes AJ (1971). Determination of saponification value of lipids. *Lab. Pract.* 20: 481.
- Kates M (1972) *Techniques of lipidology: Isolation, Analysis and Identification of lipids*. North Holland Publishing Company. pp. 359-370.
- Keith AD (1966). Analysis of lipids in *Drosophila melanogaster*. *Comp. Biochem. Physiol.* 17(4): 1127-1136.
- McHargue JS (1917). A study of the proteins of certain insects with reference to their Value as food for poultry. *J. Agric. Res.* 10: 633-637.
- Nelson DR, Sukkestad D (1968). Fatty acid composition of the diet and larvae and biosynthesis of fatty acids from carbon-14 labelled acetate in the cabbage looper. *Trichoplusia ni*. *J. Insect Physiol.* 14: 293-300.
- Onigbinde AO, Adamolekun B (1998). The nutrient value of *Imbrasia belina lepidoptera: saturnidae*(madona). *Central Africa J. Med.* 44 (5): 125-127.
- Pearson D (1976). *The chemical analysis of foods*. 7th Edition. Churchill living stone (Publisher). pp. 491-516.
- Pyke M (1979). *The science of nutrition*. In: Success in nutrition. John Murray Ltd (Publishers) London. p.175.
- Rahman SA, Huah TS, Hassan O, David NM (1995). Fatty acid composition of some Malaysian Fresh water fish. *J. Food Chem.* 54: 45-49.
- Reiser R (1973). Saturated fat in the diet and serum cholesterol concentration. A critical examination of the literature, *Amer. J. Clin. Nutri.* 26: 524.
- Rouser G, Kritchevsky G, Yamamoto A (1967). *Lipid chromatographic analysis*. Dekker Inc. New York. 11: 99-162.
- Stanley-Samuels DW, Jurenka RA., Cripps C, Blomquist GJ, Renobales MD (1988). Fatty acids in insects: Composition, metabolism, and biological significance. *Arch. Insect Biochem. Physiol.* 9: 1-33.
- Sutton MO (1988). *Insects as food: Aboriginal entomophagy in the great Basin*. Ballena Press Aththrope [Papers No 33] Ballena Press, Menlo Park, California. pp. 1-115.
- Tamaki Y (1968). Isolation of tetradecan-1, 14- dioic acid from the cornstock mealybug, *Pseudococcus comstocki*. *Lipids* 3: 186-187.
- Thompson SN (1973). A review and comparative characterization of the fatty acid compositions of seven insects orders. *Copw. Biochem. Physiol.* 45B: 467-482.
- Ukhun ME, Osasona MA (1985). Aspects of the nutrition chemistry of *Macrotermis bellicosus*. *Nutri. Rep. Int.* 32(5): 1121-1129.
- Umoh IB, Ayalogu EO, Bassir O (1980). Evaluation of the nutritive value of some lesser known protein sources in Nigerian peasant diets. *Econ. Food Nutri.* 9: 81-86

Wigglesworth VS (1976). The principles of insect physiology. 7th
Editions Methuen and Co. Ltd. (Publishers). London. p. 59.
William PC (1964). Determination of crude (total) protein using the
colorimetric method. Analyst 84: 281-283.
Yasuda M (1931). Determination of the iodine number of lipids. J. Biol.

Chem. 94: 401-409.
Zlatkis A, Zak B, Boyle AJ (1963). Determination of total cholesterol. J.
Lab. Uin. Med. 41: 486.