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Characterization of Lipids in Winged Reproductives of the Termite *Macrotermis bellicosus*

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Abstract: *Macrotermis bellicosus* (a reproductive form of winged termite) oil was extracted, and the oil was physically and chemically characterized. The lipid content of the insect was 31.46±0.57 (wet weight). The oil was a clear, odourless liquid, of a light yellow colour and it was fluid at room temperature (26±2°C). Lipid analysis revealed that the insect oil comprised 69.87±0.73% neutral lipid, 19.14±0.06% phospholipid and 10.81±0.40% glycolipids. Further analysis revealed a refractive index of 1.20±0.01, specific gravity of 0.90±0.01, solidification value of 10-14°C, total lipid phosphorus of 47.18±0.03 (mg/g lipid), acid value of 3.60±0.06, iodine value of 108.00±0.15, saponification value of 193.40±0.31, unsaponifiable matter of 12.04±0.11, free cholesterol of 8.73±1.01 (mg/100g lipid) and total cholesterol of 47.18±0.03. The unsaturated fatty acids accounted for 51.02% of the total fatty acids whereas the saturated fatty acids constituted 48.98 % of the fatty acids. These values when compared with that observed in oils which have been considered to be of high quality, suggest that *Macrotermis bellicosus* oil has potentials that could be exploited by the nutritional and pharmaceutical companies.

Key words: *Macrotermis bellicosus*, lipids, winged

Introduction

Macrotermes bellicosus simply called "Termite" in most Nigerian communities is a gregarious insect, which in Nigeria, is commonest during the rainy season.

At the onset of a rainy season, male and female long-winged reproductives fly off from their nest in large numbers. This is called 'Swarming' (Collins, 1981). During this flight, known as the nuptial (or wedding) flight, pairs of male and female termites isolate themselves from the others and fall to the ground. Their wings then break off and each pair goes its own way to form a nest in a suitable spot. They begin by making a few tunnels in the ground. In a new nest, the male reproductive is the potential king and the female, the potential queen.

In Africa, when winged termites emerge in dense numbers, they are eagerly collected. They emerge with the first rains at the ends of the dry season when people are weak from malnutrition. The termite is usually attracted to sources of illumination at nights and may be found, also, in the early hours of the mornings. The termites are harvested by placing a bowl of water under a light source. Attracted to the light they get trapped when they fall into the water. It is a highly relished food item in Nigeria, especially among children. The termite is known by various names by the different ethnic groups in Nigeria (Table 1) who strongly believe it to have high nutritive value, so they tend to recommend it to their children and pregnant women. The usual steps in the processing of the insect include dewinging, roasting and salting, or grounding into flour. They are usually consumed as part of a meal or as a complete meal with tapioca, bread, roast corn, or simply eaten as snack food. They provide important protein, fat and vitamins. The queen termites particularly are a relished delicacy. In East Africa, termite mounds are considered so important that they are owned by individuals and form part of his inheritance when he dies. (Bristowe, 1953). In Nigeria, stock cubes based on termites are easily available.

Table 1: The common names of *Macrotermis bellicosus* as known to various ethnic groups in Nigeria

Ethnic Group		Name
IBO	-	AKU
HAUSA	-	KHIYEA
YORUBA	-	ESUSUN
ESAN	-	ISEKHUN
URHOBO	-	OFURUKPE
BENIN'S (EDO)	-	EDON

With the recent wave of economic depression and its attendant effect on the purchasing power of the population of less developed nations, it has become obvious that the local food stuffs will play increasing role in the food and nutrition security of the rural people and the increasing urban poor. As popular as these termites are in Nigeria, it would appear that not much analyses have been carried out on them.

The present study was undertaken to provide data on the lipid composition of *Macrotermis bellicosus* oil as a prerequisite for the subsequent evaluation of its nutritional and pharmaceutical potential.

Materials and Methods

Sample collection: *Macrotermis bellicosus* (Termites) were obtained during their nuptial flight at Ekpoma in Edo state. They were dewinged before being used. The species were specifically identified in the Entomology Department, Nigerian Institute for Oil Palm Research (NIFOR), Benin-city, Nigeria. The insects were used within twelve hours of collection.

Solvents and chemicals used in 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 insect was extracted by the method described by Bligh and Dyer (1959). The refractive index, specific gravity and solidification values of the insect lipid were determined using the method described by the British Pharmacopia (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 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 method described by Pearson (1976) was used to determine the unsaponifiable matter as well as the acid value of the insect 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 ester (FAME) of the insect lipid was prepared using the method of Gunstone (1969). The FAME extracts were co-chromatographed with authentic FAME standards (Sigma Chemicals) 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 1.5x4mm (internal diameter) glass column of length 5 feet. The carrier gas (Nitrogen) flowed at 35ml/min while injection; oven and column temperature was 185^{0}C.

Results

Table 2 shows the total lipid extracted from *Macrotermis bellicosus*. It also shows the various lipid fractions obtained from the insect oil. The neutral lipid fraction is the major fraction in the Insect oil.

Table 3 shows the result for the physicochemical characteristics of *Macrotermis bellicosus* oil. The insect oil is a clear, odourless liquid, with a light yellow colour. It has a low solidification temperature and high iodine value indicating that the oil is unsaturated. The acid value of the oil is also low.

Table 2: Total lipid and lipid fractions in *Macrotermis bellicosus* oil

Lipid and lipid fractions	% composition
Total lipid	31.46±0.57(wet weight), 36.12±0.28(dry weight)
Lipid fractions	
Neutral lipid	69.87±0.73
Phospholipid	19.14±0.06
Glycolipid	10.81±0.40

Results represent the Mean±SEM of three estimations.

Table 3: Physicochemical Characteristics of *M. bellicosus* oil

Physical characteristics	Values
Specific gravity	0.90±0.01
Solidification value	10-14^{0}C
Refractive index	1.2±0.01
Chemical characteristics	
Acid value	3.60±0.06
Iodine value	108±0.15
Saponification value	193.40±0.31
Unsaponifiable matter	12.04±0.11
Free cholesterol (mg/100g lipid)	8.73±1.01
Total cholesterol (mg/100g lipid)	41.80±0.15
Total phosphorus (mg/g lipid)	47.18±0.03

Results represent the Mean ±SEM of three estimations.

Table 4 shows the fatty acid composition of *Macrotermis bellicosus* oil. Palmitic acid and linolenic acid are the major fatty acids in the insect oil.

Table 4: Fatty acid composition of *M. bellicosus* oil

Fatty acid	% composition
Lauric acid (C 12:0)	1.50±0.28
Myristic acid (C 14:0)	2.17±0.06
Palmitic acid (C 16:0)	42.45±0.20
Palmitoleic acid (C 16:1)	2.10±0.02
Stearic acid (C 18:0)	2.86±0.10
Oleic acid (C 18:1)	15.84±0.40
Linoleic acid (C 18:2)	24.24±1.08
Linolenic acid (C18:3)	3.90±0.60
Arachidonic acid (C 20:4)	4.94±0.15

Results represent the Mean ± SEM of three estimations.

Table 5 shows the fatty acid composition of the lipid fractions in *Macrotermis bellicosus*. The neutral lipid fraction has a fatty acid composition that is similar to that of the whole lipid. Palmitic and linolenic acids are the major fatty acids in the neutral and phospholipid fractions of the insect oil while palmitic and oleic acids are the major fatty acids in the glycolipid fraction of the insect oil.

Table 5: Fatty acid composition of lipid fractions in *M. bellicosus* oil

Fatty acids	Neutral lipid	Phospholipid	Glycolipid
Lauric acid (C12:0)	1.03±0.08	0.92±0.11	T
Myristic acid (C14:0)	2.80±0.17	4.86±0.21	1.0±0.03
Palmitic acid (C16:0)	41.54±0.90	33.15±0.40	40.03±0.60
Palmitoleic acid (C16:1)	2.09±0.28	4.10±0.11	2.14±0.15
Stearic acid (C18:0)	2.30±0.19	2.31±0.20	1.89±0.17
Oleic acid (C18:1)	11.84±0.55	13.02±0.35	35.38±0.91
Linoleic acid (C18:2)	31.42±0.25	34.14±0.45	15.20±0.20
Linolenic acid (C18:3)	3.85±0.01	2.01±0.10	4.36±0.21
Arachidonic acid (C20:4)	3.13±0.15	5.49±0.05	T

Results represent the Mean ± SEM of three estimations.

Table 6 shows the degree of saturation of the insect oil.

Table 6: Degree of saturation of *M. bellicosus oil* (% composition)

Parameter	Whole	Neutral	Phospholipid	Glycolipid
TSFA	48.98	47.67	41.24	42.92
TUFA	51.02	52.33	58.76	57.08
MUFA	17.94	13.93	17.12	37.52
PUFA	33.08	38.40	41.64	19.56

TSFA =Total saturated fatty acid. TUFA=Total unsaturated fatty acid. MUFA=Monounsaturated fatty acid. PUFA=Polyunsaturated fatty acid.

Discussion

Macrotermis bellicosus is a gregarious insect, which in Nigeria is commonest during the rainy season. The termite is usually attracted to sources of illumination at nights and may be found, also in the early hours of the mornings. It is a highly relished food item in Nigeria, especially among children. The usual steps in the processing of the insect include roasting, dewinging and salting, for use as snack food, either alone, or in combination with other foods.

The fat content of the insect is quite high when compared to values reported for a number of insects (Fast, 1970). According to Fast (1970), although fat content can reach as high as 41% of wet weight, three-fourths of known insect species contained 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 insects (Fast, 1970). The lipid content of *Macrotermis bellicosus* (Table 2) is in agreement with this statement. Fast (1967) reported a lipid value of 22.3% (wet weight) for *Rhynchophorus palmarum*. Lipid values reported for some other insects include 3.1% and 4.0% respectively for the larva and adult beetle of *Lachnosterna* species (Davis, 1918), 7.21% (dry weight) for dried melanoplus (McHargue, 1917), 2.1% for the Japanese beetle, *Popillia japonica* Newman (Fleming, 1968), 15.5% in the pupae of housefly *Musca domestica* (Calvert *et al.,* 1969). Teotia and Miller (1974) repeated the work done by Calvert *et al.* (1969) and reported the same results except that the lipid content was a little lower. 7.54% was reported for adult honey bees *Apis mellifera* L (Ryan *et al.,* 1983), 23.22% (dry matter basis) for *Anaphe venata* (Ashiru, 1988). Leung (1968, 1972) reported lipid values of 36.10, 16.94, 22.08 and 55.24 (%dry weight) for *Bombyx mori,* palm weevil larvae, crickets and termites respectively. Ekpo and Onigbinde (2004, 2005) reported lipid values of 25.30±0.20 (wet weight) and 14.87±0.33 (wet weight) for *Rhynchophorus pheonicis and Oryctes rhinoceros* larval oils respectively. Ukhun and Osasona (1985) reported a lipid value of 46.1% (moisture free basis) for *Macrotermes bellicosus*. This value is more than the 36.12% (dry weight) obtained in this study. Comparatively, the lipid value of this edible insect is higher than that found in most insects for which data is available (Fast, 1970). The fat content of this insect could have contributed to its highly acceptable flavour when fried or roasted. The lipid value of the insect 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 of protein deficiency (DeFoliart, 1992). The consumption of this insect could go a long way in taking care of the calorie needs in such communities. From the fat contents of the insect, a 100g sample will supply enough of the daily energy needs of very active people (Davidson *et al.,* 1973). This is particularly relevant in the developing countries where much energy is expended in doing works that are usually done by machines in the industrialized countries.

Available data shows that of the insects analyzed so far, 50% had a higher caloric value than soybeans, 87% were higher than corn, 63% were higher than beef, 70% were higher than fish, lentils and beans, while 95% were higher than wheat, rye or teosintle. (Phelps *et al.*, 1975; Oliveira *et al.,* 1976; Malaisse and Parent, 1980; Ashiru, 1988; Ramos-Elorduy and Pino, 1990). Table 3 shows the physical and chemical characteristics of the insect oil. The oil from *Macrotermis bellicosus* is a clear golden-yellow and odourless liquid with a solidification value of 10-14°C. This value is quite low when compared to values reported for some other oils. The iodine value (an indication of the degree of unsaturation) is quite high (108 ± 0.51). High iodine value is a common feature of most insect lipids as reported for silkworm oil 117, lepidopterous larvae between 112-159 and 108.6-118 in phytophagous *chrysomelids,* 123.6 and 140 in *Rhynchophorus pheonicis and Oryctes rhinoceros* larval oils respectively (Wigglesworth, 1976; Ekpo, 2003). The specific gravity and refractive index for *Macrotermes bellicosus* oil 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 these oils that have been considered to be of high quality and as such find much use in the pharmaceutical industries. In addition the oil is also more unsaturated than these oils, which suggests that it might 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.

The unsaponifiable fraction in the oil is 12.04±0.11 which is quite high when compared to 1.5-1.6% in *Bombyx mori* (Bergmann, 1937), 1.56% in the meal worm, *Tenebrio* cal. (Becker, 1934) and 8.60 in Rhynchophorus pheonicis larval oil (Ekpo and Onigbinde, 2004). One-third of this unsaponifiable fraction is sterol of which 85% is cholesterol (Bergmann, 1937). Sterol (especially cholesterol) is essential in insects for normal growth, metamorphosis and reproduction (Thompson *et al.*, 1973). The free and total cholesterol value for the insect oil is 8.73±1.01 and 41.80±0.15 (mg/100g lipid). These values are low compared to values reported for some conventional foods of animal origin. Ritter (1990) attributes the level of sterol in insects to species differences and diet.

Table 2 shows the lipid fractions obtained from *Macrotermis bellicosus* oil. The neutral lipid fraction was the major fraction, followed by the phospholipid fraction. This result is in agreement with observed results for other insect oils (Fast 1970; Ekpo, 2003). The neutral lipid fraction comprises mainly of triacyl glycerols, and this is an indicator of the high caloric value of the insect oil.

Gas liquid chromatographic analysis (Table 4 and 5) confirm the high level of unsaturation in the insect oil. Palmitic and linoleic (an essential fatty acid) acids are the major fatty acids in the insect oil as well as in the neutral and phospholipid fractions, while the glycolipid fraction has palmitic and oleic acids as the major fatty acids. This trend is in agreement with that observed in other insect species (Fast, 1970). As shown in Table 6, *Macrotermes bellicosus* has an unsaturation value similar to that observed for palm oil (a common household oil in Nigeria) but higher than values reported for coconut oil (Pearson, 1976). Insect fatty acids are similar to those of poultry and fish in their degree of unsaturation, with some groups being higher in linoleic and / or linolenic acids which are the essential fatty acids (DeFoliart, 1991). Nutritionally, a high level of saturated fatty acids in foods might be undesirable because of the linkage between saturated fatty acids and atherosclerotic disorders (Reiser, 1973). The presence of the essential fatty acids such as linoleic, linolenic and arachidonic acid further points to the nutritional value of the insect oil as edible oil. Niemierko (1947), observed that linoleic acid is usually formed in large amounts in larvae before pupation which suggests hormonal regulation of fatty acid synthesis One implication of the high fat content in the insect is that it may increase susceptibility of the undefatted insect 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 this insect will markedly increase the relative proportions of the other nutrients encompassed in the proximate composition. This means that greatly increased protein content can be achieved by defatting this insect.

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